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## ORIGINAL ARTICLES

# GOUT IN THE NEGRO FEMALE

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The occurrence of gout in the Negro female has heretofore been considered a rarity. The first case reported in detail appears to have been that of Perlman *et al.* in 1953[9]. The patient was a 50-year-old Negro woman who had had recurring episodes of acute arthritis for 7 years. She was found to have osseous and subcutaneous tophi, urate crystals being identified in the latter. A second patient, reported a year later by Bartfeld[1], was a 60-year-old Negress who had podagra, hyperuricemia, and roentgenographic findings compatible with osseous tophi in the metatarsal bones. Although there were no proven urate collections, the dramatic relief of acute arthritis following the administration of colchicine would appear to substantiate the diagnosis of gout. Talbott mentions examining the postmortem specimens of a Negro female with many tophi[12], and in personal communication recalls one other case of nontophaceous gout in a woman of this race[13].

Our experience, unlike that of other observers, has been that gout is not infrequent in members of the Negro race. Perhaps this is due to the large number of Negro patients seen in our clinic. We have been particularly impressed by the occurrence of typical gout in Negro women and wish to report 6 such cases. In 4 of these, urate crystals were demonstrable in biopsies of synovium or subcutaneous nodules, or both. The other 2 patients appear to have the disease in nontophaceous form. In presenting clinical and pathological data from these 6 cases, we wish to stress that gout be considered actively in the differential diagnosis of joint disease in the Negro female.

**Case Reports.** case 1. B.L., a 64-year-old Negro housewife had had recurring springtime episodes of acute arthritis for 8 years, involving the great toes, feet, knees, and the right first metacarpo-phalangeal joint. On each of these occasions, signs of inflammation persisted for approximately 10 days, and there was minimal residual discomfort of the joints.

TABLE 1.—CLINICAL DATA CONCERNIN

| *Patient* | *Age (yrs.)* | *Duration gouty arthritis (yrs.)* | *Blood pressure (mm. Hg)* | *Urine* | | |
|---|---|---|---|---|---|---|
| | | | | *Specific gravity (maximum)* | *Protein* | *Sediment* |
| Case 1, B.L.* | 64 | 8 | 140/80 | 1.017 | 5 mg./100 ml. | occas. urate crystals, epithelial casts |
| Case 2, O.F. | 61 | 11 | 240/120 | 1.007 | trace | unremarkable |
| Case 3, M.F. | 71 | 10 | 170/90 | 1.010 | 1—3+ | loaded with white cells |
| Case 4, J.G. | 52 | 14 | 110/70 | 1.018 | trace | many white cells |
| Case 5, B.S. | 41 | 3 | 200/138 | 1.012 | 1—4+ | occas. white cells, granular casts |
| Case 6, E.G.† | 52 | 6 | 156/90 | 1.023 | 0 | occas. white cells, granular casts |

*Renal function studies: glomerular filtration rate (inulin) 38 ml./min.; effective renal plasma flow (para-amin hippurate) 221 ml./min.; $Tm_{PAH}$ 69 mg./min.

†Renal function studies: glomerular filtration rate (inulin) 99 ml./min.; effective renal plasma flow (para-amin hippurate 456 ml./min.; $Tm_{PAH}$ 58 mg./min.

(These renal function studies were performed by Dr. Willoughby Lathem.)

There was no familial history of gout or rheumatism. The patient's maternal grandmother was a white woman and paternal grandmother an American Indian; both grandfathers were Negroes. Two brothers (ages 60 and 54) and one sister (age 52) were living and had no history suggestive of gout. (Serum uric acid values in these siblings were found to be normal: 5.2, 4.5, and 4.6 mg. per 100 ml.) The patient's menses had been normal and terminated at age 46. She had never succeeded in becoming pregnant.

At the time of her admission to the hospital on October 17, 1956, the patient was found to be well-developed and nourished. There were no tophi or subcutaneous nodules. Both knees were tender and moderately distended by effusions and were crepitant on motion. The ankles and the metacarpophalangeal joint of the right thumb were also swollen. There was no significant limitation in the range of motion of the joints. The remainder of the examination was not remarkable.

The urine contained less than 5 mg. per 100 ml. of protein. The retrograde pyelogram appeared normal (Table 1). The hemoglobin was 15.5 gm.%, (electrophoresis: type A), and the white cell count 12,000 per c.mm. (76% neutrophils, 6% band forms, 14% lymphocytes, 1% eosinophils and 3% monocytes). Bone marrow aspirate proved normal. Serum uric acid values were 9.4 and 9.0 mg., and blood nonprotein nitrogen 24 mg. per 100 ml. The Kolmer reaction was strongly positive. A roentgenogram of the right hand revealed narrowing of the first metacarpophalangeal space and irregular subarticular erosion of the metacarpal bone. Studies of the other peripheral articulations were normal.

Synovial fluid obtained from the left knee was light-yellow and clear. The cell count was 1400 per c.mm., of which 75% were mononuclear cells and the remainder polymorphonuclear leukocytes. Punch biopsy of the suprapatellar bursa yielded thickened synovium in which there was marked hyperplasia of the lining cells, which measured as many as ten cell layers deep. In several sections there were minute amorphous crystalline deposits of amphophilic material sur-

THE KIDNEYS AND RENAL FUNCTION

| *P.S.P. dye, % excretion* | | *Concentration of blood nonprotein nitrogen components (mg./100 ml.)* | | | *Retrograde pyelogram* | *Urine culture* |
|---|---|---|---|---|---|---|
| 30′ | 120′ | *NPN* | *Creatinine* | *Serum urate* | | |
| — | — | 24 | — | 9.0–9.4 | normal | No growth |
| — | — | 67–107 | 4–5.4 | 11.6 | small right kidney; left ureter not catheterized | — |
| 6 | 22 | 65–204 | 5.8 | 6.5–8.1 | — | *A. aerogenes; P. vulgaris* |
| 13 | 33 | 50–80 | 1.9–4.0 | 10.5–17.6 | kidney smaller than normal; loss of definition of terminal calyces | *E. coli; Staph. albus; Staph. aureus; P. vulgaris* |
| 30 | 65 | 42–60 | 2.6–5.5 | 5.6–6.8 | rt. kidney small, mod. clubbing of calyces; lt. kidney normal | Non-hemolytic streptococci |
| 55 | 80 | 15 (B.U.N.) | — | 6.3–7.5 | blunting of all major calyces and narrowing of base of upper major calyces | *E. coli* |

rounded by histiocytes. In the stroma there were numerous hyperemic thin-walled blood vessels, many of which were cuffed by a layer of macrophages and lymphocytes (Fig. 1a).

On the tenth hospital day the patient developed pain and swelling of the right thumb and both knees. She took 3 mg. of colchicine and quickly experienced marked relief. In treatment of presumed latent syphilis, she was given 14 million units of procaine penicillin, following which she again developed acute inflammation of the ankles, knees, left shoulder, and both great toes. At this time, she received phenylbutazone, followed within 24 hours by marked improvement in the arthritis.

*Comment.* The possibility of gouty arthritis was suggested by the nature of the patient's joint disease and the presence of hyperuricemia. The demonstration of urate deposits in a synovial biopsy, the roentgenographic findings compatible with an osseous tophus, and the response of an acute arthritic attack to colchicine provide clear-cut confirmation of the diagnosis of gout.

CASE 2. O.F., a 61-year-old Negro housewife, was free of joint disease until the age of 50 when she began to have intermittent podagra involving alternatingly both great toes. In the years which followed she had similar episodes of acute inflammation in the fingers, wrists, knees, and sternoclavicular joints. These attacks lasted from 3 days to 5 weeks. In the interval between attacks she was quite free of joint pain.

There was no family history of gout or rheumatism. The patient's maternal grandfather was a white man, while the other immediate ancestors were all Negro. The patient's menses had ceased at the age of 46. A single pregnancy (at age 23) had terminated in spontaneous abortion at 6 months.

When first seen in 1952 (age 56) the patient's blood pressure was 180/100 mm. Hg. There was nontender fusiform capsular swelling of the proximal joints of the fingers, and above the left olecranon process there

were several pin-head sized subcutaneous nodules. The remainder of the peripheral articulations were normal. The level of serum uric acid was 11.5 mg. per 100 ml. The urine contained 1+ protein. The hands and feet appeared normal on roentgenographic examination. Tissue taken from the left olecranon bursa disclosed a urate granuloma. The patient experienced relief of joint pain from either colchicine or phenylbutazone.

The patient failed to return for clinic visits and was not seen again until January, 1957. Flare-ups of arthritis had continued to involve the hands, feet, elbows, knees, hips and sternoclavicular joints. She returned because of "large knobs" which had developed on

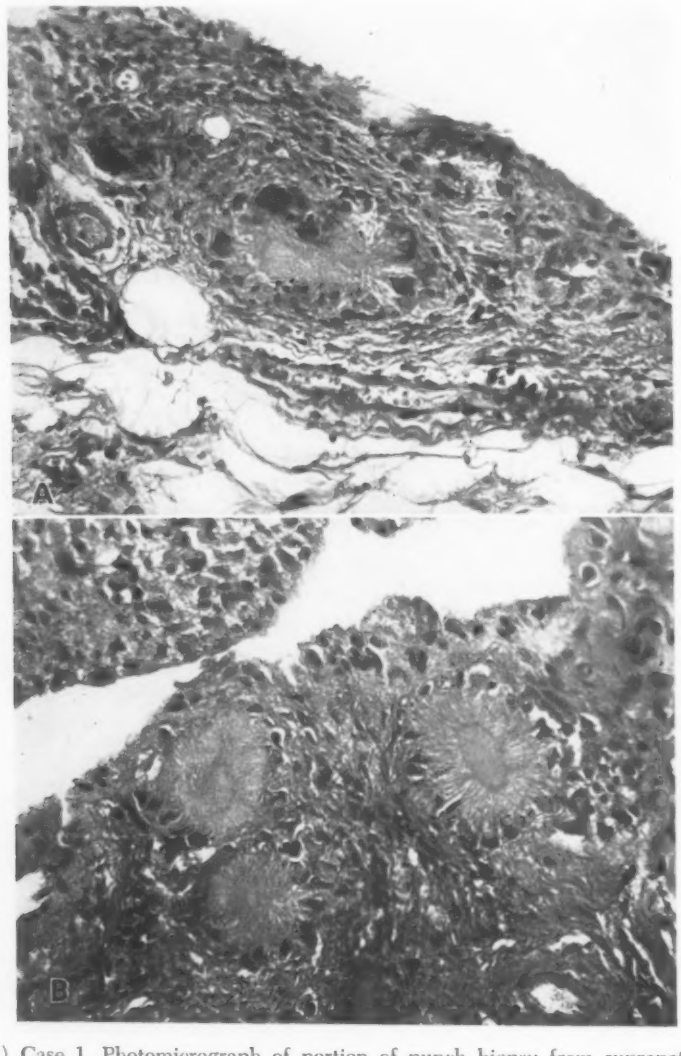


Fig. 1.—(*a*) Case 1. Photomicrograph of portion of punch biopsy from suprapatellar bursa, illustrating a small urate collection surrounded by histiocytes and giant cells in a thickened synovium infiltrated by lymphocytes and macrophages, × 280; (*b*) Case 2. Photomicrograph of portion of punch biopsy of suprapatellar bursa, illustrating urate deposits. The synovium is greatly thickened and there are infiltrations of lymphocytes and plasma cells, × 280.

her fingers in the past year, rendering her hands useless. At this time, the patient's blood pressure was found to be 240/120 mm. Hg. The mucous membranes were pallid. There were large masses on the fingers and hands, from which extruded white chalky material, identified microscopically as urate crystals (Fig. 2). The toes were similarly involved.

ential white cell counts were not remarkable. An aspirate of bone marrow was found to be hypoplastic; there were no abnormal elements present. The urine contained a trace of protein (Table 1). The level of blood nonprotein nitrogen ranged from 67 to 121 mg.; the concentration of serum uric acid was 11.6 mg. per 100 ml. Roentgenograms of the hands

Fig. 2.—Case 2. Photograph illustrating massive tophaceous deposits in hands and olecranon bursae.

The tangerine-sized left olecranon bursa was tense and fluctuant and contained several small moveable masses which were also identified as urate. Tophi were again noted above the left elbow, and now measured up to 3 cm. in diameter. The knees were enlarged and limited in motion. The remainder of the examination was not remarkable.

The hemoglobin was 6.9 gm.% (electrophoresis type A). The total and the differential white cell counts were not remarkable. An aspirate of bone marrow was found to be hypoplastic; there were no abnormal elements present. The urine contained a trace of protein (Table 1). The level of blood nonprotein nitrogen ranged from 67 to 121 mg.; the concentration of serum uric acid was 11.6 mg. per 100 ml. Roentgenograms of the hands and feet now revealed the advanced destructive changes illustrated in Figs. 3 and 4. Punch biopsy taken from the right suprapatellar bursa yielded a thickened synovial membrane containing massive deposits of urate. There was infiltration of the stroma by lymphocytes and plasma cells (Fig. 1b).

During the patient's hospital stay episodes of acute arthritis responded favorably to colchicine. She received transfusions of packed

red blood cells and experienced improvement in strength. Both olecranon bursae were excised and found to contain large amounts of urate.

*Comment.* The impression of gout, based upon the combination of typical history and hyperuricemia, was quickly corroborated by the finding of tophaceous matter in a biopsy taken from an olecranon bursa. Episodes of acute joint inflammation were relieved by colchicine. Five years later, the patient had developed massive tophaceous deposits and roentgenographic evidence of advanced destruction of bone and joint. Urate deposits were identified in both olecranon and suprapatellar bursae. At this time there was marked anemia and evidence of advanced renal disease.

Fig. 3.—Case 2. Roentgenograms of the left hand taken 5 years apart (1952–57) illustrating the marked destructive changes and tophaceous deposits which have taken place during the interim.

CASE 3. M.F., a 71-year-old Negro hairdresser, was in good health until the age of 61 when she developed the first of many recurring attacks of pain, swelling, and redness which involved at various times the knees, ankles, feet, shoulders, elbows, and hands. These attacks, while poorly described, had been severe enough to confine the patient to bed for several days to weeks. Five years after the onset of arthritis she had noted the appearance of nodules on several of the fingers. The patient had had moderate exertional dyspnea and orthopnea of several years' duration, and 2 weeks prior to admission had noted the onset of progressive swelling of the legs. There was no history of hypertension or renal disease.

The patient could give little history concerning the health or whereabouts of her par-

ents and four siblings. There was no known gout or rheumatism. Her father was a white man and her mother Negro. The patient had had a hysterectomy because of fibroid uterus at the age of 36; there were no children.

When admitted to the hospital on January 30, 1957, the patient was noted to be markedly obese. There was prominent distention of the cervical veins. Rales were heard at the bases of the lungs. The heart was enlarged. The blood pressure was 150/80 mm. Hg. There was pitting edema of the legs. Both calves were warm and tender. Firm, discrete nontender, subcutaneos nodules were noted over several of the fingers. The largest of these nodules measured approximately 1½ × 3 cm. in diameter. The peripheral joints were of normal configuration and displayed a full range of motion.

The urine contained 1+ protein. Urine cultures yielded *A. aerogenes* and later *P. vulgaris* (Table 1). The hemoglobin was 8.2 gm.%. Total and differential white cell count were normal. There was erythroid hyperplasia of the bone marrow. The concentration of serum uric acid ranged from 6.1 to 7.7 mg., and the level of blood nonprotein nitrogen was 65 mg. per 100 ml. Roentgenograms of the hands and knees revealed no sign of osseous tophi.

A punch biopsy of the right suprapatellar bursa was productive of thickened synovium in which there was marked proliferation of the mesothelial elements and a diffuse infiltration of chronic inflammatory cells, chiefly lymphocytes and plasma cells. No urate deposits were identified however. Sections of a subcutaneous nodule removed from the finger contained large irregular collections of needle-like crystalline material, pierced and surrounded by fibrous stroma, throughout which there were numerous multi-nucleated giant cells, histiocytes, and fibroblasts. This material was strikingly birefringent and was identified chemically as urate (Fig. 5*a*, *b*).

There was improvement in the dyspnea and edema when the patient was given digitalis, mercurial diuretics, and a sodium restricted diet. Three days following excision

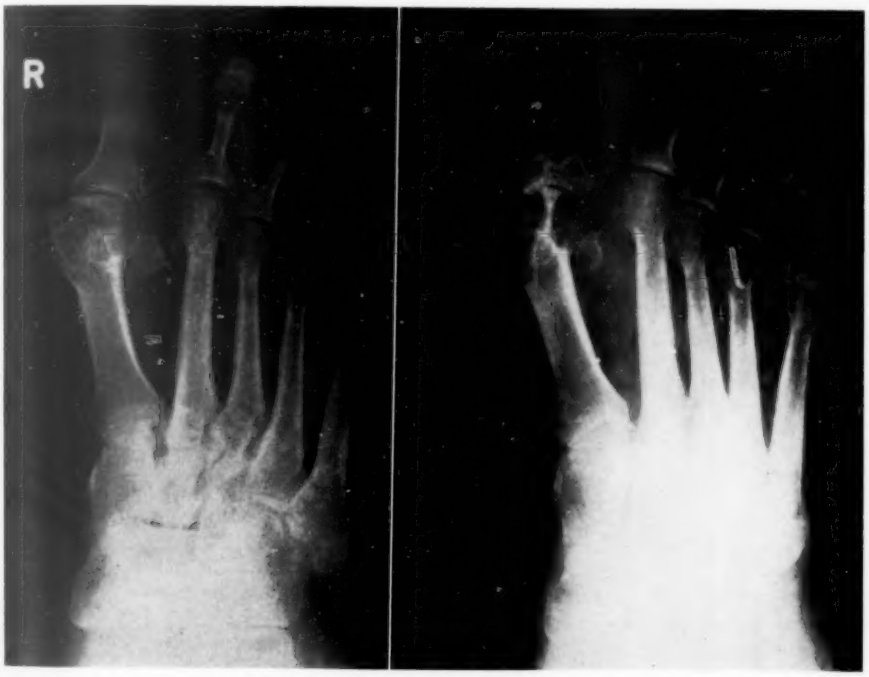


Fig. 4.—Case 2. Roentgenograms of the right foot taken 5 years apart (1952–1957). There has been striking erosion of the first metatarsal bone.

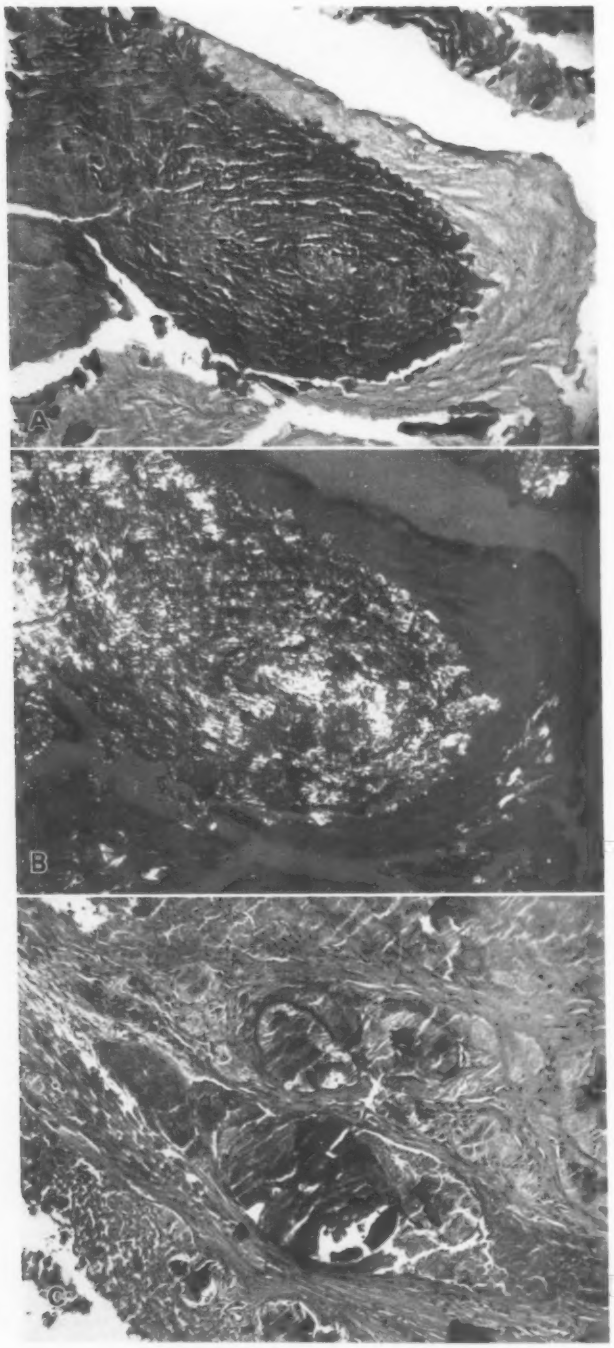


Fig. 5.—(*a*) Case 3. Photomicrograph of portion of subcutaneous nodule (tophus) removed from finger, magnification × 140; (*b*) Same section as seen under polarized light illustrating birefringency of crystalline deposits, identified chemically as urate; (*c*) Case 4. Photomicrograph of portion of biopsy from olecranon bursa stained by de Galantha technique, illustrating tophaceous deposits, × 140.

of a subcutaneous tophus, the patient's right wrist became red hot, swollen, and tender. The administration of 8 mg. of colchicine was followed by rapid and complete relief of the arthritis.

The urinary tract infection proved resistant to a variety of antibiotics. The patient became increasingly confused and delirious, and expired in uremia on the sixty-ninth hospital day. There had been gradually failing urine output, and the concentration of blood nonprotein nitrogen had risen to a high of 204 mg. per 100 ml.

*Postmortem examination.* The right kidney weighed 100 gm. and the left 120 gm. Linearly arranged white crystals were seen throughout the medullae. These were thought to be urates, but, inadvertantly, were not analyzed chemically. The principal microscopic findings were those of arteriolar nephrosclerosis and a lesser degree of chronic pyelonephritis. The parathyroid tissue was hyperplastic and there were bony changes consistent with secondary hyperparathyroidism. There was moderate cardiac hypertrophy (weight 420 gm.) and focal fibrosis of the myocardium. Atherosclerotic changes in the aorta and coronary vessels were considered slight to moderate in degree.

*Comment.* The diagnosis of gout was established in this case by the identification of urate in subcutaneous nodules which appeared 5 years after the onset of episodic arthritis. An acute attack of this latter abated quickly following use of colchicine. In the course of hospitalization there was progressive deterioration in renal function leading to death in uremia. At postmortem examination, crystalline deposits resembling urates were found in the medulla of the kidneys.

CASE 4. J.G., a 52-year-old Negro widow, was free of joint disease until the age of 38, at which time she first experienced acute arthritis in the right ankle. With gradually increasing frequency, she developed self-limited bouts of pain and swelling in other joints, including the knees, wrists, elbows, and shoulders. When seen for the first time in 1950 (age 50), she was found to have thickening of several of the proximal interphalangeal joints of the hands. The urine examination and concentration of blood nonprotein nitrogen were normal.

There was no family history of gout or rheumatic disease. The patient's menses had ended at the age of 26, following hysterectomy for "tumor."

When admitted to the hospital in December, 1951, the patient's right wrist, elbow, shoulder, and knee were swollen and exquisitely tender. There was painless fusiform swelling of several of the proximal interphalangeal joints of the hands. Pinhead sized subcutaneous nodules were felt above both elbow joints. There was marked cardiomegaly. The blood pressure was 110/70 mm. Hg. The spleen tip was felt just below the left costal margin.

The hemoglobin was 8 gm.%, and the white cell count 8,400. The differential smear contained 83% neutrophils and 17% lymphocytes. The red blood cells did not sickle. A bone marrow aspirate appeared normal. The urine contained a trace of protein and there were many white cells in some specimens. Culture of the urine yielded a variety of organisms (Table 1). The concentration of blood nonprotein nitrogen ranged from 64 to 80 mg., and of serum uric acid from 10.5 to 12.2 mg. per 100 ml. The kidneys were found to be smaller than normal in the pyelographic studies, and there was loss of definition of many terminal calyces. Areas of cystic erosion were noted in the middle phalanx of the right fourth finger, the bones of the right first metatarso-phalangeal joint, and in the tarsal bones (Fig. 6). A tissue biopsy from the left olecranon bursa revealed massive collections of crystalline material which appeared black when stained by the de Galantha technique. These deposits were surrounded by lymphocytes and foreign body giant cells (Fig. 5c).

Colchicine provided little relief of recurrent episodes of arthritis. When given 400 mg. of phenylbutazone, the patient developed edema and dyspnea, and the drug was discontinued. She then received salicylate and enjoyed marked relief of joint pain.

The patient was rehospitalized on three occasions during the ensuing year and a half because of cardiac failure, and responded poorly to treatment. She continued to have episodes of acute arthritis, and took salicylate and phenylbutazone irregularly. The concentration of blood nonprotein nitrogen ranged from 50 to 92 mg., and levels of serum uric acid reached as high as 17.6 mg. per 100 ml. She died at home on August 15, 1953. A postmortem examination could not be obtained.

*Comment.* Fourteen years after the first attack of acute arthritis, this patient was found to have subcutaneous nodules and swollen olecranon bursae, biopsy of which yielded urate-laden material, establishing the diagnosis of gout. Areas of cystic erosion were noted in bones of the hands and feet. There was azotemia of moderate degree and other evidence of renal dysfunction. In this case, episodes of acute joint pain were unaffected by colchicine, but were quickly relieved by salicylate. It is probable that a combination of cardiac and renal disease accounted for the patient's death.

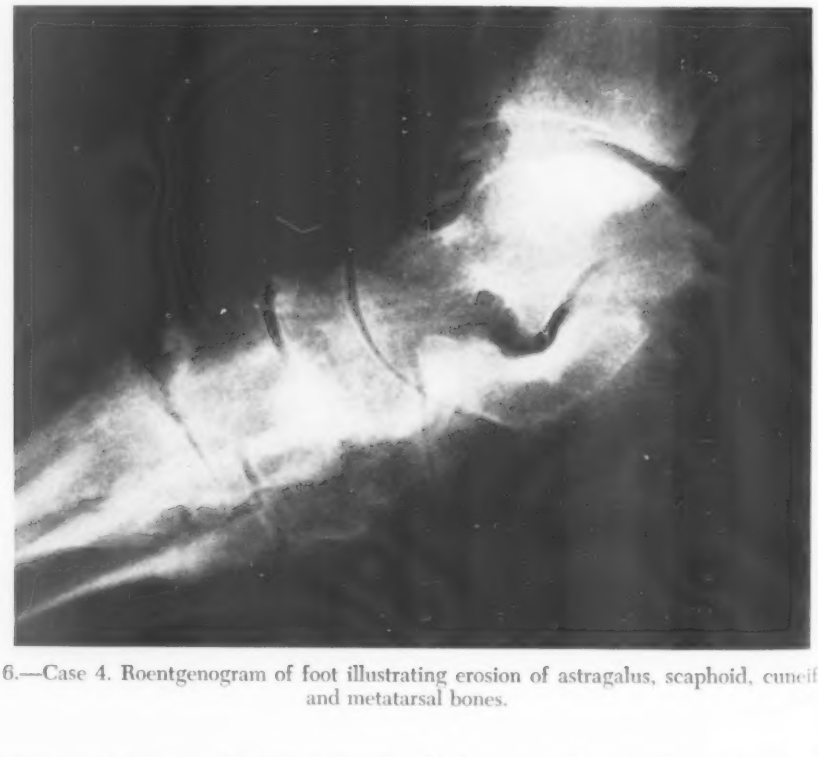

Fig. 6.—Case 4. Roentgenogram of foot illustrating erosion of astragalus, scaphoid, cuneiform, and metatarsal bones.

CASE 5. B.S., a 41-year-old Negro laboratory worker, was well until the age of 38, when she experienced the first episode of swelling, redness, heat, and pain in the ankles. The symptoms cleared in approximately one week. Within 6 months she had a recurrence of symptoms in these joints and was ill for 10 days. A third attack occurred a year later. A fourth episode in October, 1952, prompted the patient's admission to the hospital.

The patient had no siblings and no family history of gout or rheumatism. She had been found to have a positive serologic test for syphilis in 1946, and she had received "many injections." The patient's menses had begun at age 13 and had recurred regularly each 30 days. A single pregnancy, at age 18, had terminated in spontaneous abortion.

*Physical examination.* The patient was of asthenic habitus. The right pupil was smaller than the left, and neither reacted to light or in accommodation. There were no aural tophi. There was moderate cardiomegaly. The blood pressure was 200/138 mm. Hg. A nontender liver edge was palpable 3 cm. below the right costal margin. Aside from Heberden's nodes on the right index finger, the peripheral articulations appeared normal. The tendon jerks were active and there were

no pathologic reflexes. Vibratory and position sense were intact.

The hematocrit was 38% and the red blood cell count 3,800,000 per c.mm. The total and differential white blood cell counts were normal. The urine contained from 1 to 4+ protein and occasional white cells and granular casts. Cultures of the urine yielded nonhemolytic streptococci (Table 1). The concentration of blood nonprotein nitrogen ranged from 42 to 50 mg. and of serum uric acid from 5.8 to 6.8 mg. per 100 ml. The serologic tests for syphilis were strongly positive. Roentgenographic studies of the feet failed to reveal any evidence of osseous tophi.

The pupillary findings were considered indicative of central nervous system syphilis (the patient would not permit lumbar puncture), and the patient was given 30 million units of penicillin over a period of 15 days. On the day prior to completion of these injections, she developed pain and swelling of the instep of the left foot. Her temperature, which had been normal, rose to 101.6° F. and the white blood cell count to 11,800 per c.mm. The swelling of the foot progressed to involve the left great toe which became glossy, red, hot, and extremely tender. The patient was given a total of 7 mg. of colchicine and experienced complete relief of the pain and swelling. She was free of joint distress at the time of discharge. Attempts to locate the patient for follow-up study have been unsuccessful, her present whereabouts being unknown.

*Comment.* The clinical characteristics of the patient's joint disease, the presence of hyperuricemia and the dramatic reversal of podagra by colchicine are suggestive of gout. In the absence of demonstrable tophi we would consider the diagnosis presumptive but highly likely in this patient.

CASE 6. E.G., a 52-year-old Negro woman was free of joint disease until the age of 46, when she developed right-sided podagra which lasted for a month. Following this the patient was symptom free until a year later when a similar episode of painful swelling involved the right hand and wrist and lasted for two months. In the years which followed, she experienced recurrent episodes of acute inflammation involving the hands, wrists, elbows, shoulders, knees, ankles, and great toes. The joints remained normal in configuration and the only complaint in the interval between episodes of arthritis was that of slight stiffness. In 1954, the level of serum uric acid was found to be 6.9 mg. per 100 ml. Colchicine produced little relief of acute joint pain. The symptoms did respond to phenylbutazone, but this medication was discontinued because of leukopenia.

All of the patient's known ancestors were of the Negro race. Neither parent was known to have experienced gout or rheumatism. One brother, age 50, who was not available for study, is said to have arthritis, but the patient could give little detail concerning the nature of this disease. A sister, age 47, has diabetes mellitus, and was found to have a uric acid level of 3.8 mg. per 100 ml. The patient's mother and 2 older siblings had died of hypertensive vascular disease. The patient herself had had known hypertension since the age of 31. Her menses had been regular from the age of 13 until the age of 45. She had been pregnant on only one occasion and this had terminated in spontaneous abortion at 2 months' gestation.

The patient was admitted to the hospital in February, 1957. She was found to be well-developed and moderately obese. There were no aural tophi or other subcutaneous nodules. The lungs and heart were normal. The blood pressure was 156/90 mm. Hg. There was tenderness of the metacarpophalangeal joints of the right hand, as well as of the ankles and knees. The joints were of normal proportion and aside from crepitation on movement of the knees, were not otherwise remarkable.

The urine was normal (Table 1). The hemoglobin was 13.8 gm.% (electrophoresis: type A). The white blood cell count was 5,150 per c.mm., of which 53% were neutrophils, 33% lymphocytes, 13% eosinophils and 1% monocytes. The concentration of blood urea nitrogen was 15 mg., and that of serum uric acid varied from 6.3 to 7.5 mg. per 100 ml. The fasting blood sugar concentration was 105 mg.; two hours following a glucose feeding this value was 260 mg. per 100 ml. Roentgenograms disclosed narrowing of both first metatarso-phalangeal joint spaces, and erosion of the lateral articular margins and immediately adjacent sub-articular portions of both first metatarsal bones. Studies of the other articulations were unremarkable. Punch biopsy obtained from the right suprapatellar bursa yielded fragments of synovial tissue covered by a simple cuboidal mesothelium, beneath which there were concentrically thickened arterioles containing prominent hyaline deposits. There were a few small

collections of lymphocytes in the stromal tissue but no gouty deposits were found.

The patient remained free of joint pain during the course of hospitalization. Attempts to induce arthritis by the administration of such agents as crude liver extract, mercurial diuretics, and ACTH proved unsuccessful.

*Comment.* While the effect of colchicine in this case was nil, we consider gout to be the likely diagnosis because of the character of the joint involvement, persistent hyperuricemia, and roentgenographic findings suggestive of gouty erosion of the metatarsal bones. Noteworthy is the strong familial occurrence of hypertensive vascular disease and diabetes mellitus.

**Discussion.** The proportion of women in series of gouty patients has generally been limited to 5% or less (Talbott[12]), and one finds little mention of the occurrence of the disease in the Negro female. Estimates concerning the incidence of gout in different racial or other groups have little meaning, however, without reference to the population sample from which the experience is drawn and the means of case-finding employed. The 6 patients presented in this report were discovered at medical facilities in which the high percentage of Negro subjects permits more adequate detection of various disease states in this race.

While the diagnosis of gout rests most assuredly upon the demonstration of urate deposits in such tissues as synovium, tendon, and subcutaneous nodules, the combination of such features as family history of gout, passage of urate stones, hyperuricemia, characteristic arthritis, and clear-cut relief from colchicine continue to serve well in the detection of the disease in its pre-tophaceous period. In qualification of the latter criterion, however, it is to be noted that even when the drug is administered properly, as many as one-fourth of the attacks of acute gouty arthritis may prove refractory to colchicine (Bauer and Singh[2]). When first given several days after the onset of the gouty attack the effects of the alkaloid are often unimpressive indeed. It must also be emphasized that the finding of hyperuricemia does not necessarily of itself prove that the joint disease under question is of gouty origin. Patients with elevated levels of serum urate of other etiology, as well as the hyperuricemic relatives of gouty subjects, may have other types of joint disease which on occasion simulate the arthritis of gout. With these considerations in mind it can be understood why pre-tophaceous gout frequently presents a diagnostic challenge.

It is important that gout be recognized as promptly as possible, for it is in the early stages that uricosuric agents prove most effective in the prevention of chronic gouty arthritis (Gutman and Yü[7]). Since patients are required to remain under treatment for a number of years, if not a lifetime, it would appear desirable that confirmation of the diagnosis be sought by means of examination of suitable biopsy material for pathognomonic urate collections. In this regard, we have found punch biopsy of the suprapatellar bursa a useful procedure (Zevely *et al.*[15]). Specimens of synovium obtained by use of the Polley-Bickel needle[10] frequently yield tophus-studded tissue in which urate deposits are identified in characteristic granulomata composed of focal collections of crystalline material surrounded by histiocytes, giant cells, and lymphocytes. On occasion flecks of urate are visible on macroscopic examination of the specimen. In tissue sections the deposits of urate appear birefringent under polarized light, and they may be further identified by use of appropriate staining technique (de Galantha[4]). It has been our practice to fix a portion

of all tissue biopsies in absolute alcohol to limit the dissolution of water-soluble urate, but we have found that sufficient tophaceous material may be recognized *in situ* even when 10% formalin has been used as a fixative. While the finding of urate collections is considered *prima facie* evidence of gout, failure to observe these focal deposits does not by any means eliminate the diagnosis. We have obtained synovial biopsies from subjects with typical acute gouty arthritis in which inflammatory changes were marked, but no urate could be identified. In other cases, for example Case 1, the synovial tophi were sparse in number and required careful search of many tissue sections before discovery.

Four of the 6 women reported above were found to have tophaceous deposits in the synovium of suprapatellar bursae (Cases 1 and 2), olecranon bursae (Cases 2 and 4), or subcutaneous nodules (Case 3). These patients all had a history of recurrent arthritis clinically suggestive of gout (including 2 with podagra), and in this group we would consider the diagnosis of gout to be indisputable. In the case of patients B.S. and E.G. (Cases 5 and 6), both of whom experienced podagra, the clinical characteristics of the joint disease appear sufficiently suggestive to warrant the diagnosis of gout, despite the lack of detectable tophaceous deposits.

In 2 patients (Cases 1 and 5), episodes of typical gouty arthritis followed upon the administration of large amounts of penicillin, a substance well known for its provocation of the gout (Talbott[12]).

In none of these 6 patients did the clinical findings or hematologic studies suggest secondary gout (Gutman[6]). While we believe, that in all, the gout is of the primary variety, in no case was there a familial incidence of the disorder. However, in only 2 instances could the family history be considered accurate in this regard. Four siblings available for study (Cases 1 and 6) all had normal levels of serum urate.

The patients themselves were all found to have elevated serum urate concentrations. The values noted in this group ranged from 6.8 to 17.6 mg. per 100 ml. The average level (Table 1) would seem to be somewhat lower than the peak incidence of 9 to 10 mg. per 100 ml. found in a group of gouty males (Gutman and Yü[7]). It has been noted that there is a sex difference in respect to serum urate levels in both normal individuals and in those with "essential" hyperuricemia (Gutman and Yü[5], Stecher, Hersh and Solomon[11], and Wolfson *et al.*[14]). Using a method employing the enzyme uricase, Yü and Gutman found a mean serum urate level of 5.4 $\pm$ 1.4 mg. per 100 ml. in a group of normal men, and a significantly lower 4.1 $\pm$ 0.9 mg. in a group of normal women[5]. In the study of the heredity of gout and its relationship to familial hyperuricemia, Stecher and co-workers observed a significant correlation between the emergence of hyperuricemia and the age of the affected female relatives of gouty subjects[11]. Not only was the elevation of serum urate concentration limited to the postmenopausal years, but the degree of hyperuricemia was less marked than that in affected males. In only one of the patients herein reported (Case 5) did gouty arthritis appear during the period of active menstrual function; in this case the first attack of arthritis occurred at age 38, at which time the patient continued to have regular menses. All in all, however, there were only 4 attacks of gout in the next 3 years.

In 3 of the 4 patients from whom the information was available, one or more progenitors were not of the Negro

race. The maternal grandmother of patient B.L. (Case 1), the maternal grandfather of patient O.F. (Case 2), and the father of patient M.F. (Case 3) were all whites, while the paternal grandmother of patient B.L. (Case 1) was an American Indian. Such interracial mixture is not uncommon in urban Negro populations. None of the parents of our patients was available for study (almost all having long since expired) so that it is not possible to assess the relative genetic contributions of the Negro vs. the non-Negro ancestors to the development of gout in the offspring. It is possible that transmission of the gouty trait occurred in some via a non-Negro ancestor.

It may be of some interest to note that none of the 6 patients bore progeny. There were a total of only 3 pregnancies, each of which terminated in spontaneous abortion. While the fertility of gouty women appears to be low (Talbott[12]), that of women with hyperuricemia alone seems to be normal, according to the observations of Stecher *et al.*[11].

All 6 patients in this group were found to have some abnormality of renal function or structure (Table 1). In 2 (Cases 2 and 3), there was frank renal insufficiency, and the latter of these patients expired in uremia. There were a variety of combinations of abnormal findings. In Case 1 there was severely restricted glomerular filtration and renal plasma flow despite normal urinalyses, blood nonprotein nitrogen concentration, and retrograde pyelogram. In Cases 2 and 4 relatively minor disturbances in the urine examination were associated with moderate to marked azotemia and markedly abnormal pyelograms. In Case 5 proteinuria was at times marked, azotemia slight to moderate in degree, and only the right kidney found to be abnormal in the pyelographic study. Lastly, in Case 6, while the urine examination and the excretion of phenolsulfonphthalein and other indices of renal function were within normal limits, an intravenous pyelogram disclosed calyceal blunting and other changes.

Considering the group as a whole, the most striking features are the frequency of urinary tract infection (Table 1) and the number with abnormal pyelograms. The kidneys of 4 of the patients were described as small and there was blunting or clubbing of the calyces: alterations suggestive of chronic pyelonephritis. To this may be added the finding of pyelonephritis and arteriolar nephrosclerosis in the postmortem study of Case 3.

Many observers have commented upon the marked variability of the pathologic changes in the kidneys of gouty subjects, the diversity and marked chronicity of the clinical characteristics of the associated renal dysfunction, and the fact that the severity of the structural alterations may be unrelated to the clinical course of the disease (Talbott[12]). There is disagreement concerning the pathogenesis of this renal disease but there can be little doubt that the deposition of urates either as calculi or as crystals in the tubules and interstitial tissues must ultimately prove damaging, and that superimposed pyelonephritis will cause further compromise of renal function (Fineberg and Altschul[3] and Lichtenstein, Scott and Levin[8]).

**Summary.** The clinical and pathological findings of gout are reported in a group of 6 Negro women. The patients ranged from 41 to 71 years in age, and had had recurrent arthritis for periods of 3 to 14 years. In 4 instances the diagnosis of gout was confirmed by the demonstration of tophaceous deposits in synovial biopsies or subcutaneous nodules, or both. In 2

cases the diagnosis seemed warranted on clinical grounds, despite the lack of detectable urate collections. In none was there evidence to suggest that the gout was of the secondary type.

Renal disease of varying degree was noted in each of the 6 patients. One woman died in uremia and was found to have arteriolar nephrosclerosis and pyelonephritis. The kidneys were reduced in size and there were crystalline deposits in the medullae.

Punch biopsy of the suprapatellar bursa has proven to be a valuable procedure in furnishing pathognomonic evidence for the diagnosis of gout. Synovial tissue is frequently found to contain telltale urate deposits.

In presenting these cases of gout, we would emphasize that estimates of the incidence of a given disease in various racial groups have little meaning without reference to the population sample from which the experience is drawn, and the methods and criteria employed in diagnosis. In the case of gout it appears that the disease is not as rare in the Negro female as has been generally considered. The diagnosis should be considered actively in *all* patients presenting appropriate symptoms of the disease.

ADDENDUM: We have recently observed a seventh Negro female, aged 60, who had had two episodes of podagra in a period of 3 months. The patient was known to have had arterial hypertension for many years. There was no family history of gout. The maternal grandmother was white. Both great toes and adjacent dorsum of the feet were found to be swollen, red, and tender. The patient's temperature was 101° F. The concentration of serum uric acid was 7.0 to 9.2 mg. and of blood urea nitrogen 19 mg. per 100 ml. No osseous tophi were evident on roentgenographic examination of the feet. The administration of 4 mg. of colchicine was followed promptly by subsidence of fever and marked relief of joint pain. We believe these findings indicative of gouty arthritis.

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*(Interlingua on page 382)*

# TRANSFORAMINAL CURET NECROPSY OF BRAIN: A NEW APPROACH TO POSTMORTEM TISSUE DIAGNOSIS WITH CASE HISTORY AND DISCUSSION

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The occasional reluctance of families toward granting permission for the classical necropsy has led to the development of the needle necropsy using Vim-Silverman[2] needles or the Murray-Franklin modification for obtaining tissue from liver, kidney, spleen, prostate, lung, and other organs. These readily accessible organs are available for needle punctures to obtain specimens which, while not entirely adequate, usually suffice for the diagnosis of generalized disease of these organs (Silverman[2] and West and Chomet[3]).

The brain is the organ which is most difficult to study without a head necropsy, and most frequently necropsies of this organ are limited or prevented by specific request of surviving next of kin, or excessive alopecia which prevents easy masking of the prosectorial incision.

This problem has presented itself on occasion at the Omaha Veterans Administration Hospital and we have made an attempt at a partial solution which is satisfactory in generalized brain disease, and may, with serendipity be as useful in localized brain lesions.

Many of the older German pathologists were adept at removing small segments of brain tissue through the vault of the mouth and through small trephined holes in the skull. These approaches are of necessity limited by ease of opening the jaw and the difficulties in trephining, closing incisions and burr holes to prevent postmortem leakage of embalming, bloody and serous fluids.

The position of choice is the lateral decubitus with head anteflexed upon the chest in order to widen the useful aperture of the foramen magnum as much as possible. The head is maintained in anteflexion by an assistant while the pathologist inserts the guide needle of the Craig vertebral body curet[1] through the foramen magnum in an anterocephalad direction. The curet cannula is slipped over the guide needle and advanced into the cisterna (Fig. 1). The guide needle is removed and cerebral spinal fluid is aspirated from the cisterna. When an adequate

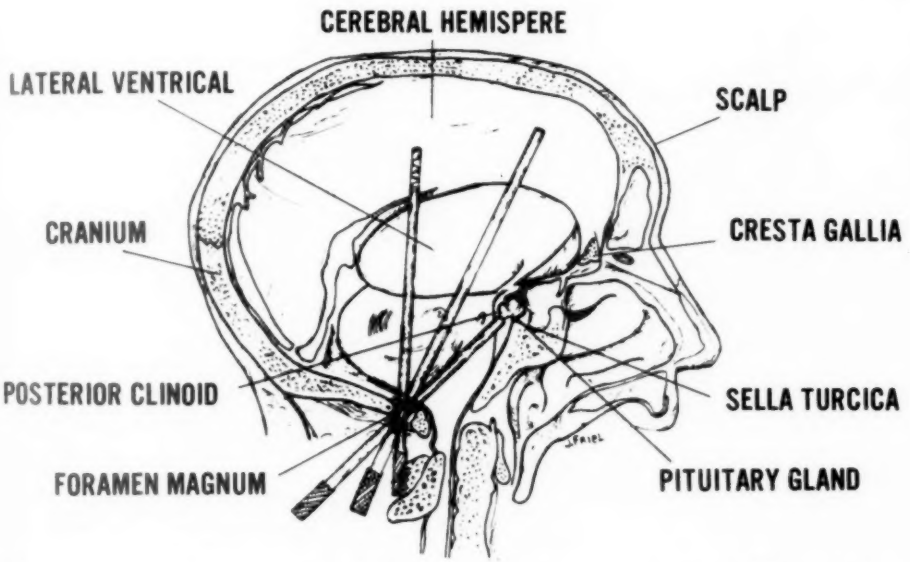


Fig. 1.—Needle necropsy approach to brain and meninges by foramen magnum approach using the Craig vertebral bone curet.

The method we have found satisfactory, transforaminal curet necropsy of the brain, has many advantages: simplicity, ease of positioning the body, adequate tissue and cisternal fluids obtainable for histological, biochemical and bacteriological examination. The leakage of embalming fluids is also prevented.

**Method.** The body preferably should not be embalmed prior to this procedure as embalming fluid tends to set the muscles of the neck and prevents anteflexion to obtain the best approach to cerebral areas. This procedure does not interfere with embalming of the brain or scalp.

sample has been obtained a clean syringe is attached and the needle advanced to the right and inferiorly to enter the right lateral ventricle. Specimens of brain tissue from mid-brain, right cerebral hemisphere, and right pia arachnoid and dura are obtained by toothed and hooked cutters inserted within the lumen of the cannula, and syringe aspiration. The cannula is withdrawn to the cisterna and inserted into the left mid-brain, left lateral ventricle and meninges for cutter biopsy. This technique is repeated in other areas of the brain until representative samples of all brain tissue except cerebellum and frontal lobes are obtained. The neck can not be flexed sufficiently anteriorly to direct the needle into the cerebellum from this position. The petrous ridges and posterior clinoids

protect the frontal lobes from this approach. A short wooden dowel, 1½ inches long and 5/16 inch in diameter, is sharpened in a pencil sharpener and driven into the curet hole effectively to seal off the opening to foramen magnum.

**Case Report.** HISTORY: A 63-year-old white male billing clerk was admitted to the hospital on July 5, 1957, for the eighth hospital admission with the chief complaints of weakness, fever, weight loss, band-like headaches and stiff neck for one month. He stated that he had been under treatment by a radiologist for chronic lymphocytic leukemia. A sternal puncture and cervical node biopsy on his second admission here revealed a chronic lymphatic leukemia. Treatment for the leukemia had been Roentgen-ray therapy, and his blood count was within normal limits until June, 1955, when he had a 19,050 leukocyte count. In December of the same year there was an increase to 49,000, and several months later to 100,000 leukocytes, almost all of which were lymphocytes. Since that time he has had minimal doses of radiation therapy, 100 roentgens per treatment, over the spleen to a total of 1700 roentgens.

PHYSICAL EXAMINATION: Weight 153½ pounds, temperature 101°, pulse 88, respirations 18, blood pressure 120/70. He was a well-developed, somewhat emaciated white male, who appeared to be in acute distress from headache. There was marked enlargement of the cervical lymph nodes bilaterally which had a soft, almost fluctuant texture. Ophthalmoscopic examination was normal. The lungs were clear. The liver extended 5 cm. below the right costal margin and was nontender. The spleen was felt 5 cm. below the left costal margin. There were numerous nodes palpated through the abdominal wall, varying from 2 to 5 cm. in size. Abdominal palpation produced slight tenderness but no rebound tenderness. The prostate was moderately enlarged. The hands showed palmar erythema. Enlarged lymph nodes were present in the axillary, epitrochlear, femoral and inguinal areas. Neurological examination was normal except for moderate nuchal rigidity and a positive Kernig's sign.

LABORATORY AND ROENTGENOLOGIC DATA: Red blood cell count was 3,300,000 with 10.5 gm. of hemoglobin, leukocytes numbered 163,200 with 1 stab, 1 neutrophil, 97

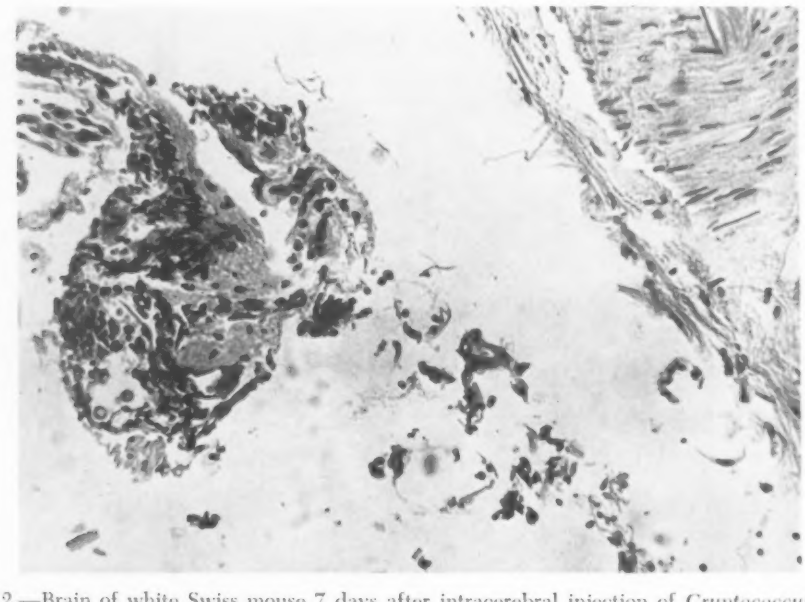

Fig. 2.—Brain of white Swiss mouse 7 days after intracerebral injection of *Cryptococcus neoformans* from spinal fluid of W.H.S. Presence of *Cryptococcus neoformans* in pia arachnoid can be seen. One organism is budding. Heavy round cell reactive infiltrate is present. The adjacent nerve trunk appears to be unaffected.

lymphocytes and 1 eosinophil per h.p.f. The peripheral smear contained many immature lymphocytes, including occasional blast forms and many smudge cells. Total protein was 6.9, albumin 4.4, globulin 2.5 gm.%. Platelet count was 108,000. VDRL test was negative. Liver function tests, including Bromsulphalein, cephalin flocculation and van den Bergh, were normal. Urinalysis was normal.

*Pathological Examination.* The bone marrow revealed many immature lymphocytes and few megakaryocytes. A lymph node biopsy showed the stroma to be replaced almost entirely by lymphocytes with little pleomorphism. Stains for fungi were done but none were found. Urine culture and sensitivity tests reveal Proteus organisms sensitive to chloramphenicol, streptomycin and nitrofurantoin.

*Tissue Examination.* Brains of 4 mice were injected intracerebrally with suspension of organisms from the spinal fluid of patient obtained from culture on brain heart infusion blood agar. Sections through the mouse brains showed typical *Cryptococcus neoformans* organisms present within the leptomeninges (Fig. 2).

COURSE IN THE HOSPITAL. The patient was treated with potassium iodide and a triple sulfonamide without benefit. Five days later he was started on Mycostatin. He had received transfusions for anemia. Because the leukocyte count was 376,000 he was given chlorambucil 6 mg. daily. The platelet count fell to 54,000 per c.mm. The patient continued to deteriorate. He developed a pneumonitis in the left lung field associated with an increasing temperature which terminally rose to 104° rectally, and he became comatose. He suddenly expired, 25 days after admission.

ANTIBIOTIC SENSITIVITY STUDIES. The organism (*Cryptococcus neoformans*) cultured from the patient's spinal fluid was found to be sensitive *in vitro* to sulfamerazine, nitrofurazone and nystatin at therapeutic levels. The yeast was resistant to penicillin, chlortetracycline, streptomycin, tetracycline, chloramphenical, erythromycin, oxytetracycline, nitrofurantoin, oleandomycin, novobiocin, sulfadiazine and sulfamerazine.

Further studies were done with Amphotericine B* (Fungazone) on *in vitro* sensitivity. The Cryptococcus was found to be resistant *in vitro* to levels of 0.2 mg. per ml. but sensitive to the inhibitory effect of 0.4 mg. per ml. and higher concentrations of Amphotericine B. These were within the therapeutic levels.

THE NEEDLE NECROPSY. Permission for a complete postmortem examination was refused but the family authorized a needle necropsy (West and Chomet[3]). The tissues were obtained from the kidney, liver, lung and pleura, and prostate by use of both Vim-Silverman[2] and Murray-Franklin modifications by the usual approaches. Tissues obtained were adequate for histological diagnosis. The inadequacy of conventional needle necropsy of the brain (West and Chomet[3]), led us to develop, through modification, a special technique for obtaining intracranial tissue.

HISTOLOGICAL FINDINGS. *Brain.* The arachnoid is edematous, congested, and contains numerous lymphocytes. Scattered through it are Cryptococci. Occasional budding forms are seen. Foci of adjacent cerebral cortex are involved and show hemorrhage, necrosis, and scattered organisms. The vessels throughout the brain are congested. Occasional organisms are seen in the cerebrum adjacent to vessels. There are leukemic infiltrates and scattered foci of necrosis in the cortex (Fig. 3).

*Liver.* The central and portal veins are dilated and the sinusoids are congested. Many of the hepatic cells show cloudy swelling and some contain brown pigment. The sinusoids and triads contain a leukemic infiltrate.

*Kidney.* The cortex contains numerous hyalinized glomeruli. Many of the tubules contain hyaline and protein casts. The interstitial tissue and many of the capillaries contain leukemic cells.

*Prostate.* The prostate has an abundant fibromuscular stroma containing a leukemic infiltrate. The glands are lined by atrophic epithelium and many contain corpora amylacea.

*Lung and Pleura.* Were cultured and found to be positive for *Cryptococcus neoformans.*

*Amphotericine B (Fungazone) was generously supplied by E. R. Squibb & Sons.

**Discussion.** The needle necropsy, while not as exhaustive in its findings as the classical necropsy, may be adequate to solve many problems of pathological and etiological nature, when the usual necropsy is denied. In this case, useful tissues were obtained from brain, meninges, lung, liver, kidney, prostate, which were useful for both microscopic tissue diagnosis and culture.

The transforaminal curet necropsy of brain appears to be adequate for the study of generalized brain disease and has the advantage of supplying larger fragments of tissue for study than the ordinary aspiration biopsy.

**Summary.** 1. A modified technique for transforaminal curet necropsy of brain and meninges is described which provides more adequate tissue specimens than the Vim-Silverman needle technique. This may prove useful in cases where routine necropsy is refused or where alopecia prevents routine necropsy of skull.

2. This approach was valuable in confirming the diagnosis of cerebral cryptococcosis in a patient with lymphomatous disease. Needle necropsy provided adequate tissue for culture and microscopic examination from major accessible organs of the body.

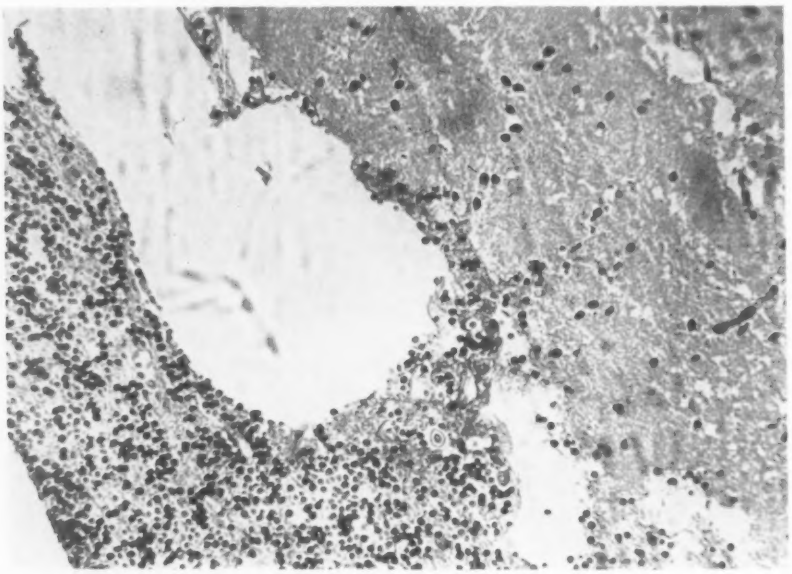

Fig. 3.—Needle necropsy of brain and meninges. The arachnoid is edematous, congested and contains numerous lymphocytes. Scattered through the arachnoid are organisms containing dark central areas surrounded by clear areas. Occasional budding forms are seen. Foci of the adjacent cortex are involved and show hemorrhage, necrosis and leukemic infiltration.

ACKNOWLEDGMENTS: Illustrations are by the Medical Illustration Service of the Omaha Veterans Administration Hospital. The authors wish to express their appreciation for the kind assistance of Dr. Robert Grissom, Professor and Director of the Department of Medicine, University of Nebraska College of Medicine.

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## SUMMARIO IN INTERLINGUA

### Necropsia Cerebral Per Curettage Transforaminal: Un Nove Methodo De Histodiagnose Necroptic, Con Le Historia De Un Caso E Discussion

1. Es describite un technica modificate pro le necropsia del cerebro e del meninge per medio de curettage transforaminal. Illo produce plus adequate specimens histologic que le technica a agulia de Vim-Silverman. Le nove methodo promitte provar se utile in casos in que le necropsia routinari es refusate o in que le extreme alopecia del cranio prohibi su inclusion in le necropsia routinari.

2. Le nove technica se ha monstrate de valor in confirmar le diagnose de cryptococcosis cerebral in un patiente con morbo lymphomatose. Le necropsia a agulia provideva adequate specimens histologic pro objectivos de cultura e de examine microscopic ab le major accessibile organos del corpore.

## SUMMARIO IN INTERLINGUA

*(See page 290 for original article)*

### Vaccino De Influenza Asian: Effecto Del Etate Del Vaccinato E Del Regime Del Vaccination Super Le Responsa Al Antigeno

1. Es presentate datos relative al responsa anticorporee a vaccination con un vaccino monovalente de influenza asian, basate super un studio de 312 individuos.

2. Le individuos in iste studio e in plus 250 personas in le personal del hospital qui recipeva vaccinationes ab le mesme lot de vaccino esseva tenite sub observation con respecto a possibile effectos lateral. Minus que 3% de iste individuos exhibiva grados significative de local o systemic effectos lateral.

3. Individuos de plus que 40 annos de etate, prendite como gruppo total, monstrava un melior responsa al vaccination a dose unic que individuos de minus que 40 annos de etate.

4. Immunisation per 2 doses, con un intervallo de 4 septimanas inter le doses, esseva superior tanto al doses unic como etiam al duple doses con un intervallo de 2 septimanas, quando le dose de vaccino monovalente usate esseva 200 unitates de agglutination de cellulas de gallina.

5. Le via intracutanee del inoculation esseva inferior al via subcutanee quando le vaccino esseva administrate como un sol injection a individuos sin previe experientia con le antigeno. Tamen, quando administrate como secunde dose de un plano de immunisation a 2 doses con un intervallo de 4 septimanas, le via intracutanee e le via subcutanee produceva responsas de character comparabile.

6. Le disponibilitate del racia asian pro le incorporation in un vaccino polyvalente rende possibile un considerabilemente plus large spectro de protection contra influenza epidemic.

# ASIAN INFLUENZA VACCINE: EFFECT OF AGE AND SCHEDULE OF VACCINATION UPON ANTIGENIC RESPONSE

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It has been demonstrated in the past with other strains of influenza that a high level of protection can be afforded by the use of killed influenza vaccines, if the specific strain involved in the epidemic has been incorporated into the vaccine and if sufficient antigenic mass has been administered at the proper time relative to exposure. Experience with other types of influenza has indicated that a 200 chick cell agglutinating (CCA) unit dose of vaccine when given to a population without prior experience with the antigen results in a relatively poor initial antibody response (Davenport[1]). This primary experience with the antigen may, however, make possible an adequate booster response to a second dose of vaccine given several months later. Early studies had indicated that almost the total population was without demonstrable antibody to this new strain of virus. At the time this study was conceived, it seemed that one might not be able to afford the luxury of time to pursue the previously outlined schedule of two doses several months apart because of the imminence of clinical influenza. Actually there was no information available to indicate that the optimal schedule for vaccination with the new monovalent 200 CCA unit Asian influenza vaccine was the same as that established for other strains. This study attempts to assess: a) The effect of a single dose primary immunization upon antibody titer, b) the effect of a 2-dose primary immunization upon antibody titer, c) the efficacy of 1.0 ml. subcutaneously compared to 0.1 ml. intracutaneously in eliciting an antibody response. (Such schedules have been proposed in the past by some as being interchangeable and a smaller volume requirement recommends itself in times of vaccine shortage.) And d) the effect of age of the recipient upon the antibody response to vaccination.

**Methods.** The methods of serologic testing have been described in detail (Boger and Liu[2], Jensen[8]). Certain points are worth restating here. The factors discussed above will be assessed on the basis of the hemagglutination inhibition titer (HAI), which is specific for the Asian type of influenza. This test system, which employs the Japan/305A strain at its 18th to 19th passages in chick embryos, did not give nonspecific reactions even in the absence of special treatment of the serum with cholera filtrate or potassium periodate. This statement is based upon our experience of a consistent finding of titers of $<2$ in over 600 persons under the age of 70 years who had not had vaccination or

Asian influenza infection in 1957. The reciprocal of the highest dilution which produced a complete inhibition of the agglutination of a 1% chicken red blood cell suspension was considered to be the titer of antibody against the Asian virus antigen. All titers in this paper refer to the initial dilution of the patients' serum. During the addition of equal volumes of virus and chick red cell suspension in the performance of the test the serum is further diluted. For those who prefer to consider the final dilution of serum values given here must be multiplied by three.

for higher titers in the 40 to 70-year-old age group. In the chi-square type of statistical analyses employed, the number of values $<2$ and $>2$ were compared for the age groups 17 to 40 and 40 to 70 years of age. To facilitate visual inspection of the data, a vertical dashed line in Figs. 1 and 2* will indicate this point of separation in the chart and also provide an index of those patients who were living at the

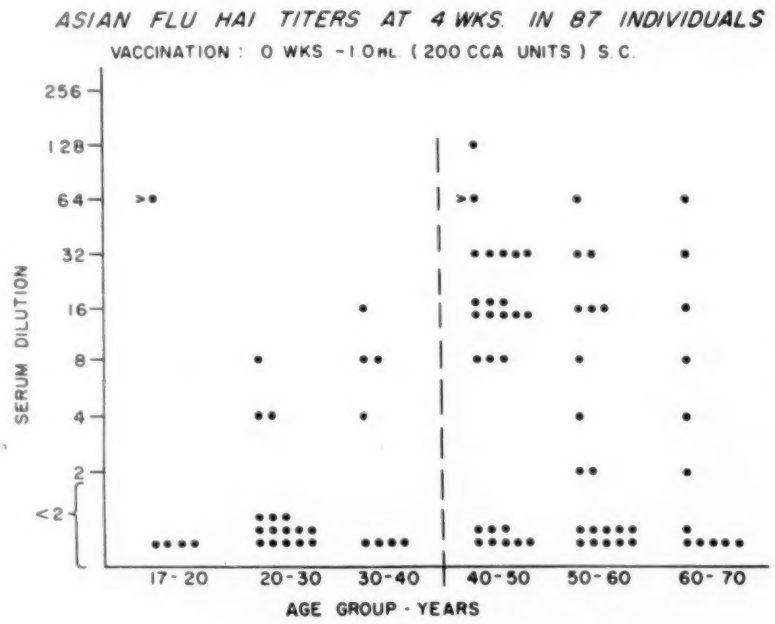


Fig. 1

The individuals vaccinated in these studies were staff personnel or institutionalized patients who would be a high risk when the epidemic reached the hospital.

Blood specimens were obtained from antecubital vein and allowed to clot at room temperature and to retract in the refrigerator over night. The serum was harvested into 10-ml. sterile vials and held at −4° C. until submitted for titration.

**Results.** AGE EFFECT. The results reported in Figs. 1 to 3 show a tendency

time of the 1918 epidemic. The probability that this distribution of titers in the older group might have occurred due to chance is $<1$ in 50 for the data presented in Fig. 1, $<1$ in 1,000 for the data in Fig. 2, and $<1$ in 50 for Fig. 3. With respect to Fig. 3, the data at 4 weeks were chosen for the analysis. This effect of age upon antibody response has been presented at the out-

*The authors are indebted to Dr. Hamilton Eaton of the Harrisburg State Hospital, Harrisburg, Pa., for his cooperation in supplying blood specimens from this group of patients.

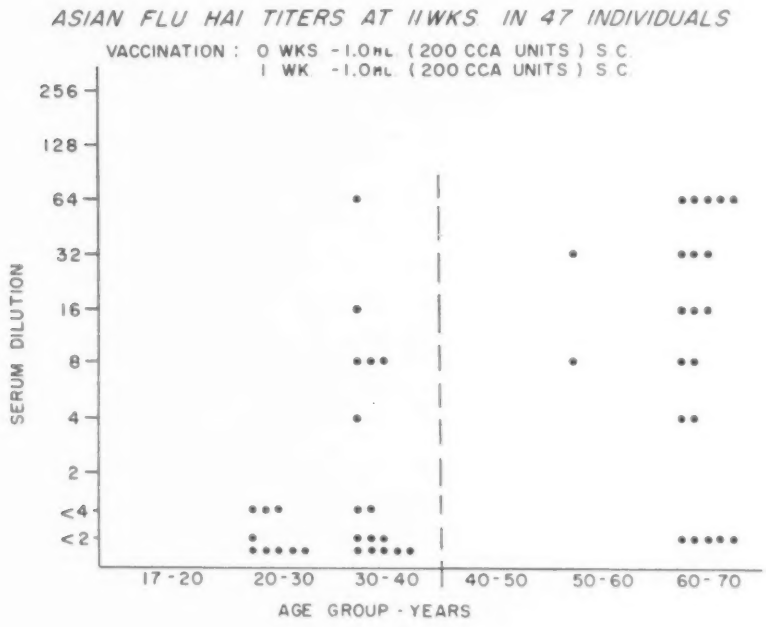
ASIAN FLU HAI TITERS AT 11 WKS IN 47 INDIVIDUALS
VACCINATION : 0 WKS - 1.0 ML (200 CCA UNITS) S.C.
1 WK - 1.0 ML (200 CCA UNITS) S.C.
SERUM DILUTION
256
128
64
32
16
8
4
2
<4
<2
17-20
20-30
30-40
40-50
50-60
60-70
AGE GROUP - YEARS

Fig. 2

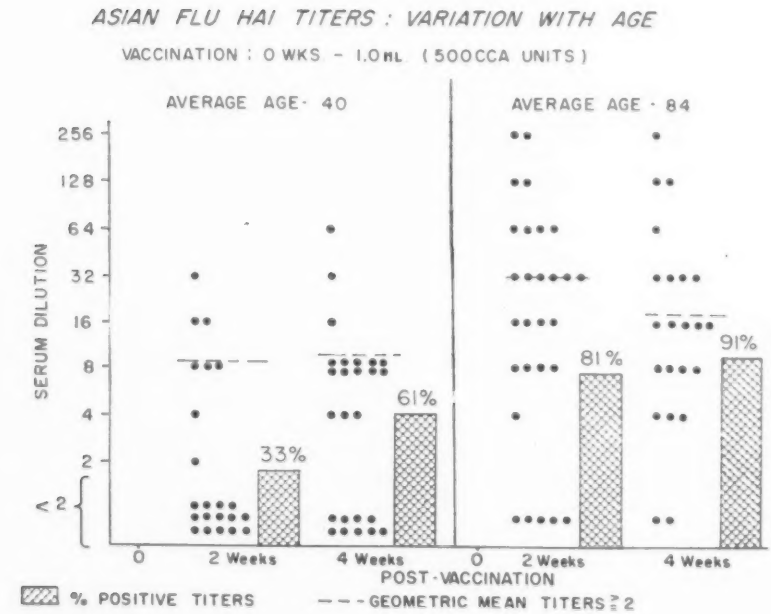
ASIAN FLU HAI TITERS : VARIATION WITH AGE
VACCINATION : 0 WKS - 1.0 ML (500 CCA UNITS)
AVERAGE AGE - 40
AVERAGE AGE - 84
SERUM DILUTION
256
128
64
32
16
8
4
2
< 2
33%
61%
81%
91%
0
2 Weeks
4 Weeks
POST-VACCINATION
% POSITIVE TITERS
GEOMETRIC MEAN TITERS ≥ 2

Fig. 3

set in order to emphasize that subsequent comparisons of antigenic response were carried out in groups of similar age and that this was important.

EFFECT OF MULTIPLE DOSE SCHEDULES UPON ANTIGENIC RESPONSE. As a corollary to the evaluation of multiple dose schedules, it seemed purposeful to determine the time at which the maximum incidence of positive titers or the maximum height of the titer, or both, occurred after a single injection. Accordingly, studies with sampling at 0, 2 and 4 weeks postvaccination and at 0, 4 and 6 weeks were initiated. In each instance the vaccination schedule was 200 CCA units (1.0 ml. subcutaneously) given on one occasion. The data are presented in Figs. 4 and 5 and suggest that both the absolute titer and the per cent of individuals with positive titers were still rising at 2 weeks and declining at 6 weeks. In contradistinction to this rather mild response to the single exposure to 200 CCA units of inactivated virus, we have observed high sustained titers following naturally occurring disease, that is, 100% of individuals with positive titers and a geometric mean of 1 to 64 at 8 weeks after infection (Boger, Bayne and Liu[1]).

van Gelder, Greenspan and Du Fresne[7] in 1947 and Bruyn, Meikeljohn and Brainerd in 1949[8] using vaccines containing strains with which the population had had prior antigenic experience describe comparable antigenic responses when the subcutaneous route of administration was compared to one-tenth of the subcutaneous dose given intracutaneously. It seemed of interest to inquire as to what results might be obtained if this were reinvestigated with Asian influenza, a strain with which the present popula-

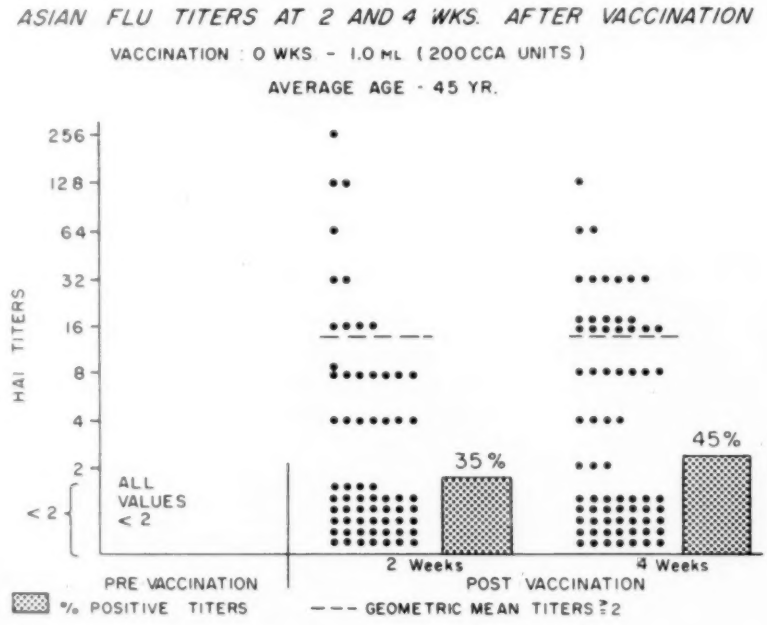


Fig. 4

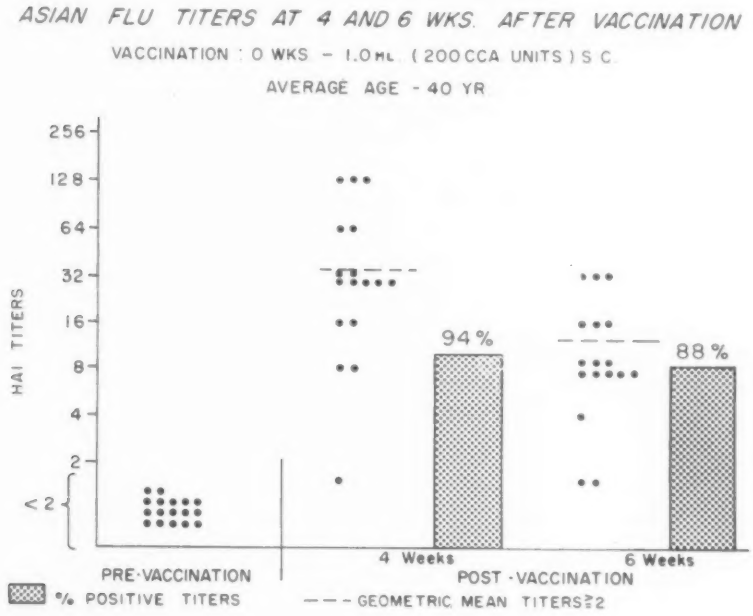
ASIAN FLU TITERS AT 4 AND 6 WKS. AFTER VACCINATION
VACCINATION : 0 WKS – 1.0 ML (200 CCA UNITS) S.C.
AVERAGE AGE - 40 YR
HAI TITERS
256
128
64
32
16
8
4
2
< 2
94 %
88 %
4 Weeks
6 Weeks
PRE-VACCINATION
POST-VACCINATION
% POSITIVE TITERS
GEOMETRIC MEAN TITERS ≥ 2

Fig. 5

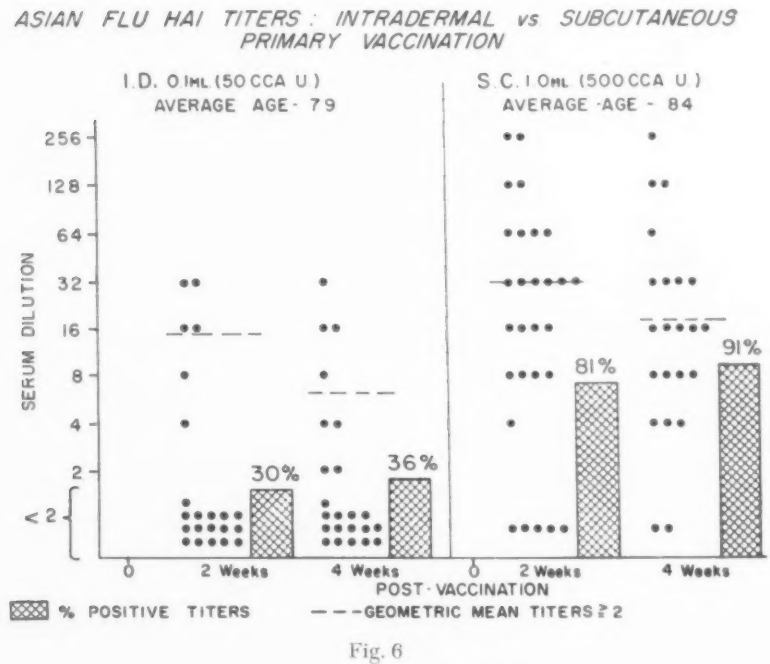
ASIAN FLU HAI TITERS : INTRADERMAL vs SUBCUTANEOUS PRIMARY VACCINATION
I.D. 0.1 ML (50 CCA U.)
AVERAGE AGE - 79
S.C. 1.0 ML (500 CCA U.)
AVERAGE AGE - 84
SERUM DILUTION
256
128
64
32
16
8
4
2
< 2
30%
36%
81%
91%
0
2 Weeks
4 Weeks
POST-VACCINATION
% POSITIVE TITERS
GEOMETRIC MEAN TITERS ≥ 2

Fig. 6

tion has had very little, if any, prior experience. In a previous publication[2], we have presented data supporting the opinion that 0.1 ml. intracutaneously is probably not optimal as the primary immunization (Fig. 6). Despite this, it seemed worth further exploration to ascertain whether the intracutaneous inoculation was of value as a second injection.

A study involving 5 groups of 50 individuals each was undertaken in an attempt to assess whether a second injection should be given at a 2- or 4-week interval and, also, whether one could distinguish between 0.1 ml. intracutaneously as contrasted to 1.0 ml. subcutaneously. The five schedules which were compared are described in Table 1. The same lot of Asian influenza vaccine was used throughout.* Since each schedule was to be assessed on the basis of at least two post-vaccination antibody determinations, it seemed important to consider only the titers of individuals on whom data were complete at all sampling periods. This rigid criterion resulted in the elimination of considerable data; however it seemed necessary to eliminate the possibility of bias due to incomplete sampling in small groups. By so doing it was hoped that the interpretation that changes in mean titer were related to time after vaccination would be strengthened.

The individual titers, the geometric mean of the positive titers, and the percentage of individuals having titers equal to or $>2$ are presented for each schedule in Figs. 7 to 11. It will be noted that the average age for these 5 groups is comparable. From consideration of the data one sees that a second injection in 2 weeks had little influence, whether it was 1.0 ml. subcutaneously or 0.1 ml. intracutaneously. A second dose at 4 weeks enhanced the antibody response and there seemed to be no significant difference at this interval between the intracutaneous versus the subcutaneous route of vaccination.

TABLE 1.—SCHEDULES OF IMMUNIZATION
Influenza Virus Vaccine—200 CCA Units*

| | *Weeks After Immunization* | | |
|---|---|---|---|
| | *0* | *2* | *4* |
| Group I | 1.0 ml. S.C. | | |
| Group II | 1.0 ml. S.C. | 1.0 ml. S.C. | |
| Group III | 1.0 ml. S.C. | | 1.0 ml. S.C. |
| Group IV | 1.0 ml. S.C. | 0.1 ml. I.C. | |
| Group V | 1.0 ml. S.C. | | 0.1 ml. I.C. |

*Lot #40175 B–1

**Discussion.** In view of the rather transient nature of the antibody rise to vaccinations, it seems likely that the practice of at least two vaccinations during the winter season is as necessary for the Asian strain as it is for other strains currently incorporated in the polyvalent influenza vaccines. This is, of course, presumption in the absence of data on higher unitage vaccine or with booster injections.

Francis[6] has in the past proposed that influenza virus has but a finite

*Lot #40175 B–1 supplied through the courtesy of Merck Sharp & Dohme Laboratories, West Point, Pennsylvania.

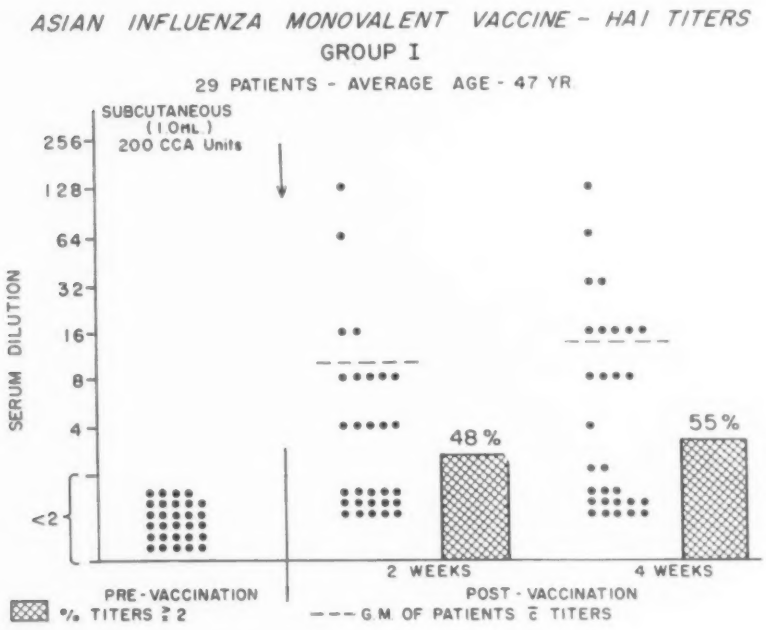
ASIAN INFLUENZA MONOVALENT VACCINE - HAI TITERS
GROUP I
29 PATIENTS - AVERAGE AGE - 47 YR
SUBCUTANEOUS
(1.0ML)
200 CCA Units
SERUM DILUTION
256
128
64
32
16
8
4
<2
48%
55%
2 WEEKS
4 WEEKS
PRE-VACCINATION
POST-VACCINATION
% TITERS ≥ 2
G.M. OF PATIENTS ≥ TITERS

Fig. 7

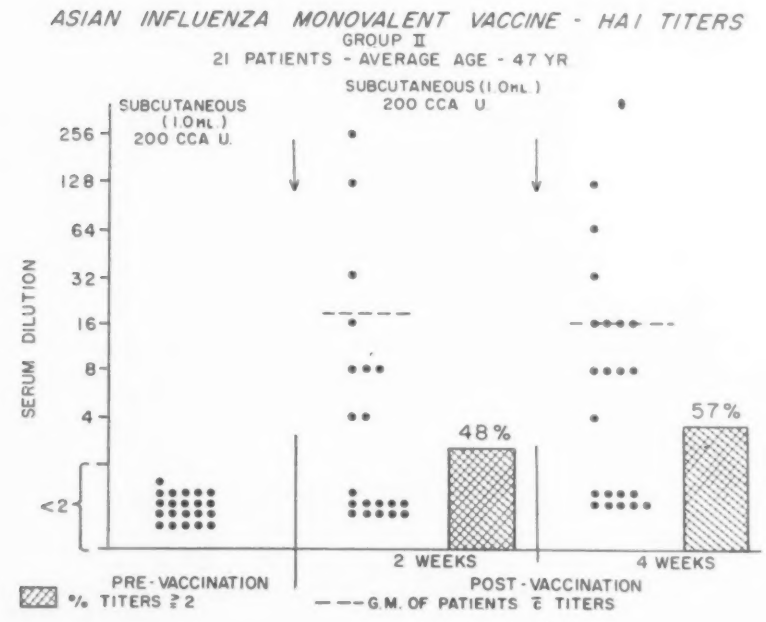
ASIAN INFLUENZA MONOVALENT VACCINE - HAI TITERS
GROUP II
21 PATIENTS - AVERAGE AGE - 47 YR
SUBCUTANEOUS (1.0ML)
200 CCA U.
SUBCUTANEOUS
(1.0ML)
200 CCA U.
SERUM DILUTION
256
128
64
32
16
8
4
<2
48%
57%
2 WEEKS
4 WEEKS
PRE-VACCINATION
POST-VACCINATION
% TITERS ≥ 2
G.M. OF PATIENTS ≥ TITERS

Fig. 8

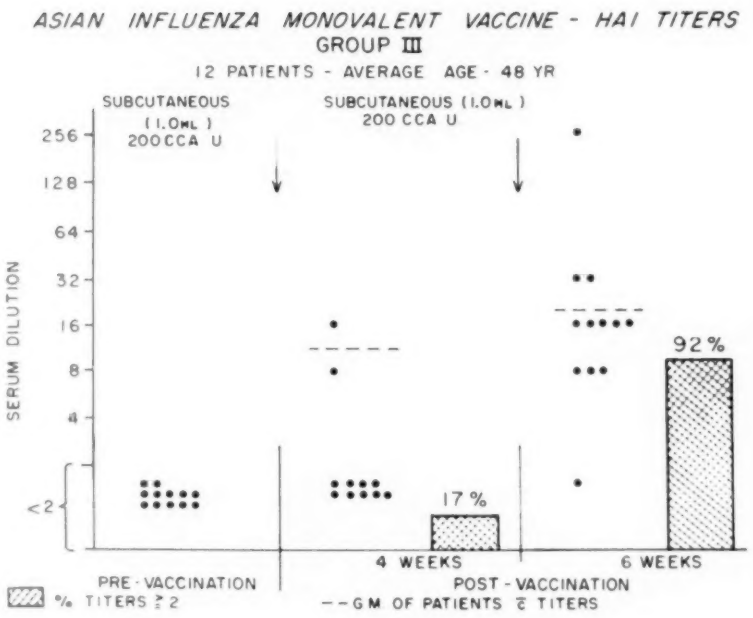
ASIAN INFLUENZA MONOVALENT VACCINE - HAI TITERS
GROUP III
12 PATIENTS - AVERAGE AGE - 48 YR
SUBCUTANEOUS (1.0ML) 200CCA U
SUBCUTANEOUS (1.0ML) 200 CCA U
SERUM DILUTION
256
128
64
32
16
8
4
<2
17 %
92 %
4 WEEKS
6 WEEKS
PRE-VACCINATION
POST-VACCINATION
% TITERS ≥ 2
--GM OF PATIENTS ≥ TITERS

Fig. 9

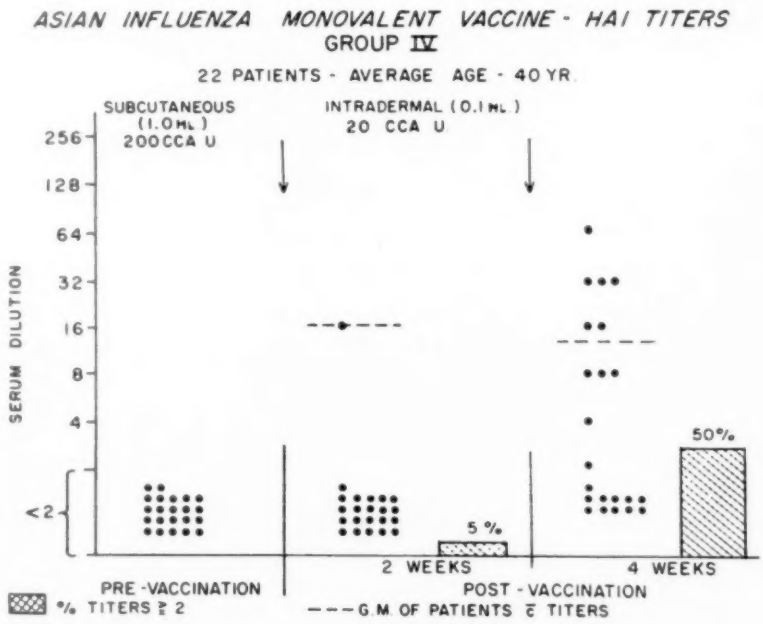
ASIAN INFLUENZA MONOVALENT VACCINE - HAI TITERS
GROUP IV
22 PATIENTS - AVERAGE AGE - 40 YR
SUBCUTANEOUS (1.0ML) 200CCA U
INTRADERMAL (0.1ML) 20 CCA U
SERUM DILUTION
256
128
64
32
16
8
4
<2
5 %
50%
2 WEEKS
4 WEEKS
PRE-VACCINATION
POST-VACCINATION
% TITERS ≥ 2
---G.M. OF PATIENTS ≥ TITERS

Fig. 10

number of antigenic variations available to it and that sometime it might come full circle, returning to the antigenic composition of earlier strains. We are quite intrigued by a report from Mulder[9] that he was able to demonstrate antibodies against the Japan/305A strain in the sera from older individuals who would have been alive at the time of the 1888–90 influenza epidemic which also originated in Asia.

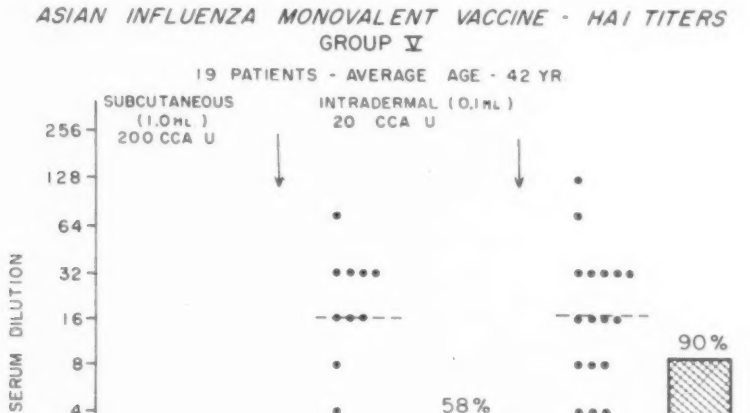


Fig. 11

We have confirmed this observation in our own hospital population in patients over the age of 70 years[2] using a type of assay which has repeatedly shown titers $<2$ in over 600 individuals age 19 to 70 when not exposed to influenza or vaccination. Further, using a 1956 commercial vaccine which did not contain the 1957 Asian strain, we were able to elicit antibodies to Japan/305A virus in 3 of 25 (12%) of individuals over 70 years of age so treated. We are inclined to believe that these findings are related to the reinforcement of antibody against the virus to which the individual received his primary experience in his youth as discussed by Davenport, Hennessy and Francis in 1953[5]. It is difficult to infer from the observed enhanced response to vaccination in older individuals whether this is simply related to multiple exposure to influenza in this older group or whether this is indicative of shared antigenic characteristics between the earlier type A virus strains and the Asian strain.

If, in fact, the Type A influenza virus has exhausted its possibilities for antigenic variation, this is of considerable importance. Vaccination is the only present form of protection against influenza and in the past it has been very difficult to prepare sufficient vaccine to protect the population during

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It will be interesting to observe by what transpires in the future whether the control of epidemic influenza is at hand or whether what we have reported in this paper and referred to above is nothing more than the sharing of some antigenic characteristic between 1957 Asian influenza, 1888–90 Asian influenza, swine influenza, and perhaps PR8 influenza as well.

**Summary.** 1. Data have been presented from the study of 312 individuals relative to their antibody response to vaccination with a monovalent Asian influenza vaccine.

2. The individuals in this study, as well as an additional 250 persons on the staff of the hospital who received this same lot of vaccine, were followed as regards side effects. Less than 3% of these individuals exhibited significant local or systemic side effects.

3. Individuals over the age of 40 years, as a group, had a better response to a single dose vaccination than did persons under the age of 40.

4. A 2-dose immunization with a 4-week interval between doses was superior to either a single dose or 2 doses 2 weeks apart, when a 200 chick cell agglutinating unit monovalent vaccine was employed.

5. The intracutaneous route of inoculation was inferior to the subcutaneous route when given as a single injection to individuals without prior experience to the antigen. However, when administered as the second dose of a 2-dose immunization schedule with a 4-week interval, the intracutaneous route and subcutaneous routes gave comparable responses.

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## REFERENCES

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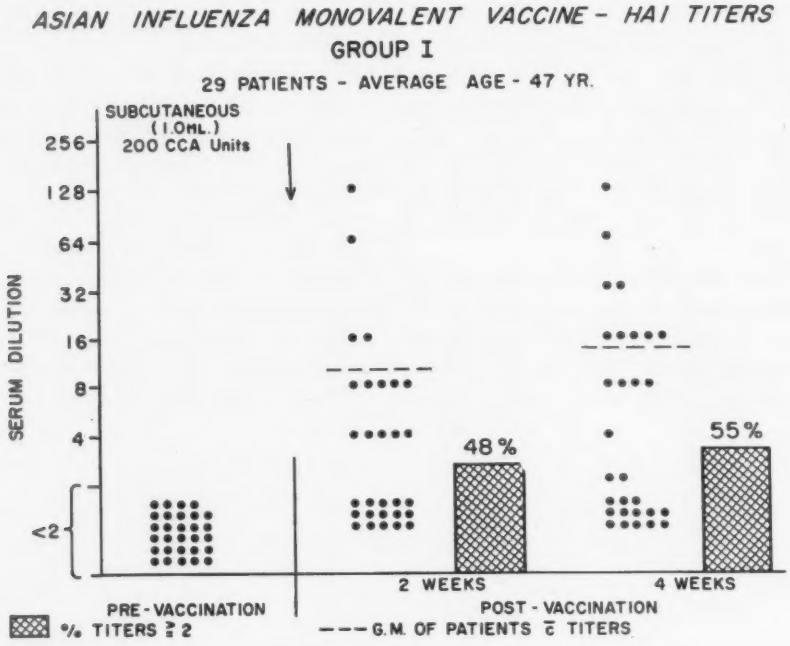
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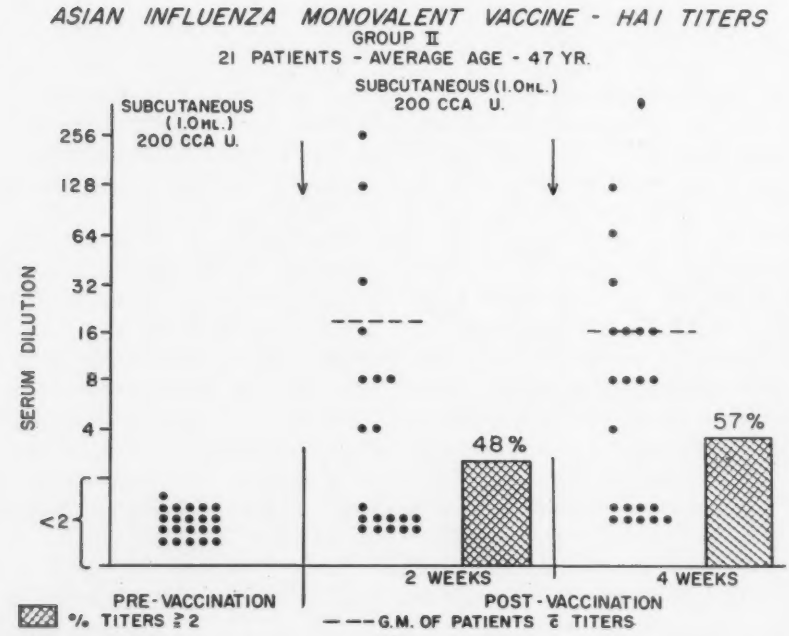
GROUP I
29 PATIENTS - AVERAGE AGE - 47 YR.
SUBCUTANEOUS (1.0ML.) 200 CCA Units
SERUM DILUTION
256
128
64
32
16
8
4
<2
48%
55%
2 WEEKS
4 WEEKS
PRE-VACCINATION
POST-VACCINATION
% TITERS ≥ 2
G.M. OF PATIENTS ē TITERS

Fig. 7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GROUP II
21 PATIENTS - AVERAGE AGE - 47 YR.
SUBCUTANEOUS (1.0ML.) 200 CCA U.
SUBCUTANEOUS (1.0ML.) 200 CCA U.
SERUM DILUTION
256
128
64
32
16
8
4
<2
48%
57%
2 WEEKS
4 WEEKS
PRE-VACCINATION
POST-VACCINATION
% TITERS ≥ 2
G.M. OF PATIENTS ē TITERS

Fig. 8

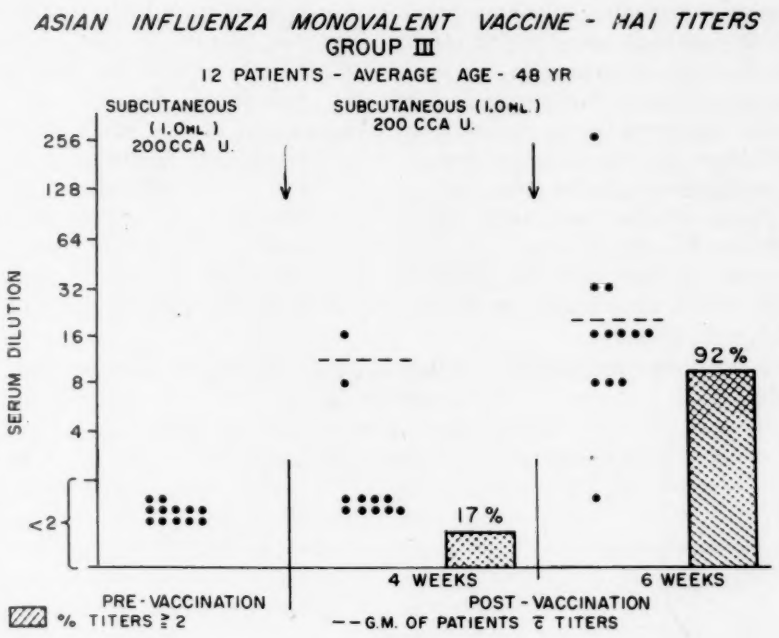


Fig. 9

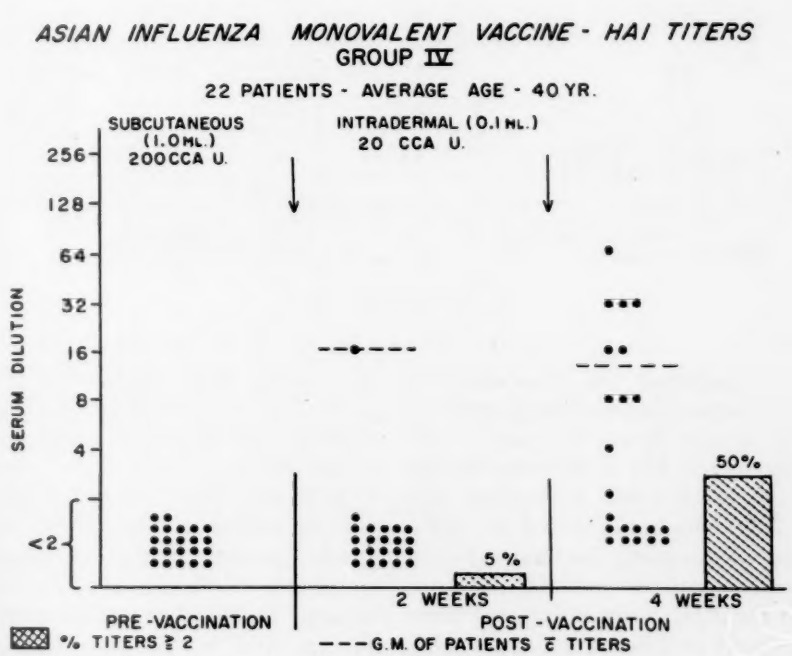


Fig. 10

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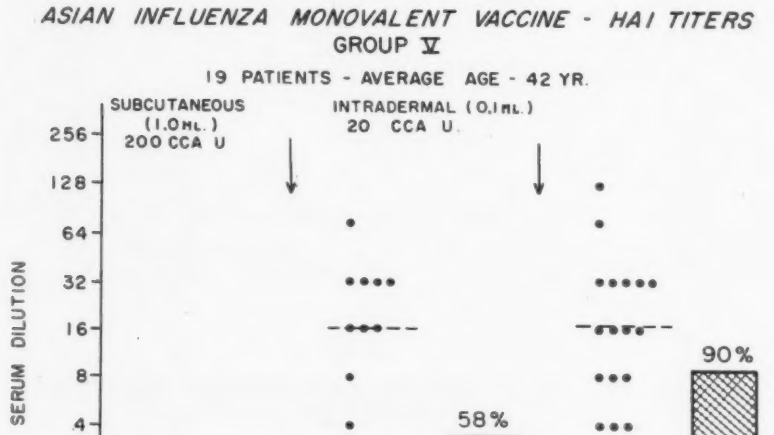


Fig. 11

We have confirmed this observation in our own hospital population in patients over the age of 70 years[2] using a type of assay which has repeatedly shown titers <2 in over 600 individuals age 19 to 70 when not exposed to influenza or vaccination. Further, using a 1956 commercial vaccine which did not contain the 1957 Asian strain, we were able to elicit antibodies to Japan/305A virus in 3 of 25 (12%) of individuals over 70 years of age so treated. We are inclined to believe that these findings are related to the reinforcement of antibody against the virus to which the individual received his primary experience in his youth as discussed by Davenport, Hennessy and Francis in 1953[5]. It is difficult to infer from the observed enhanced response to vaccination in older individuals whether this is simply related to multiple exposure to influenza in this older group or whether this is indicative of shared antigenic characteristics between the earlier type A virus strains and the Asian strain.

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9. **Mulder, J.:** Lancet, **2,** 334, 1957.

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# MEPHENTERMINE AND THE ARRHYTHMIAS

By Michael Wilson, M.D.

Michael Perez-Arzola, M.D.

and

M. J. Oppenheimer, M.D.

(From the Department of Physiology, Temple University School of Medicine, Philadelphia, Pennsylvania)

Cardiac arrhythmias continue to constitute a serious therapeutic problem (Soloff[9]). Ectopic impulse formation in the ventricle may, under the influence of anesthesia and surgical manipulation, develop into ventricular fibrillation. It has been said that antifibrillatory agents, therefore agents to abolish ectopic impulse formation, should have their major effect in prolonging the refractory period with a minor decrease in conduction velocity (Meek[4]). On the other hand, a member of the pressor amine group of drugs, mephentermine sulfate, has been proved to be an effective antifibrillatory agent (Lynch, Webber and Oppenheimer[3]). The mechanism of this protective action is due to a direct myocardial influence. The fundamental properties of conduction velocity and refractory period are changed (Stewart *et al.*[10]). Velocity of conduction is accelerated, that is, conduction time is shortened. The refractory period is shortened. Therefore, it becomes evident that in the pharmacological modification of arrhythmias (Moe[5]) alternate explanations are possible.

With these effects upon the myocardial parameters of conduction velocity and refractory period, it would seem possible to favorably influence other cardiac arrhythmias. Indeed, this has been shown to be true in experimentally produced arrhythmias in the dog (Oppenheimer *et al.*[7,8]).

The cardio-salutary actions of mephentermine sulfate have been demonstrated in the dog (Oppenheimer, Lynch and Barrera[8]). With this experimental evidence, new concepts for the therapeutic approach of arrhythmias have been advanced. The need for new anti-arrhythmic drugs is evident (Wiggers[11]).

The purpose of this clinical study is to evaluate the acute response of various cardiac arrhythmias in the abnormal heart, to mephentermine sulfate.

**Material and Methods.** Twelve patients were studied, 8 males from 52 to 80 years and 4 females from 24 to 36 years of age. One was studied on two different occasions.

Seven had arteriosclerotic heart disease; 3, hypertensive heart disease; 1, active rheumatic fever; and 1, a congenital complete heart block. Six were in chronic congestive heart failure and one had had a recent myocardial infarction. Nine were receiving digitalis; one was considered to have digitalis toxicity.

After a 12-lead electrocardiogram and after a long tracing of the lead that showed best either the P wave or the arrhythmia, mephentermine sulfate* was given intravenously. Subsequent long tracings were taken after each administration of mephentermine sulfate and from 10 minutes to 4½ hours after completion of total dosage. In some cases electrocardiograms were taken the next day.

Table 1.—Effect of Mephentermine on Patients in Group 1.

| *Basic Rhythm & Rate* | *Frequency Ectopic Impulse Formation* | *Other Effects* |
|---|---|---|

*Available as Wyamine Sulfate, Wyeth Laboratories, Philadelphia, Pennsylvania.



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TABLE 1.—EFFECT OF MEPHENTERMINE ON PATIENTS IN GROUP 1.

| Subject | Age | Sex | Diagnosis | Receiving Digitalis | Total Dose mg. | Dose/kg. Body Wt.—mg. | Basic Rhythm & Rate | | Frequency Ectopic Impulse Formation | | Other Effects | | Duration of Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Control | Max. Effect | Control | Max. Effect | Objective | Subjective | |
| A.N. | 80 | M | Hypertensive Cardiovascular Disease | No | 98 | 1.4 | Sinus Radial 44/min Apical 88/min | Sinus 94/min Radial & Apical Rate = 92 min | Bigeminus | PVC 15/100 Systoles | No significant BP Change | None | 4% 25 mins |
| C.K. | 71 | M | Chronic Congestive Heart Failure | Yes | 90 | 1.5 | Sinus 75/min | Sinus 120/min | 24/100 Systoles | PVC 2.5/100 Systoles | BP rise of 40 mm. Hg. Incr. Respir. Rate | Slight apprehensiveness; Sense of warmth | 37.5% 2 hours |
| I.W. | 36 | F | Active Rheumatic Fever | Yes | 60 | 1.0 | Sinus Apical 100/min | Sinus 100/min | Bigeminus | PVC 12/100 Systoles | No BP Change | Mild Nervousness. Desire to void | 0% 1 hour |
| I.W. | 36 | F | Active Rheumatic Fever | Yes | 120 | 2.0 | Sinus Apical 100/min | Sinus 100/min | Bigeminus | PVC 0/100 Systoles | BP Rise of 8 mm. Hg. | Sense of Nervousness; Desire to void | 0% 1 hour |
| Bkz. | 70 | M | Chronic Congestive Heart Failure Arteriosclerotic | Yes Toxicity? | 52.5 | 0.75 | Atrial Tachycardia Atrial = 187/min Ventricular = 144/min | Atrial Tachycardia Atrial = 185/min Ventricular = 185/min | 44/100 Systoles | PVC 0/100 Systoles | No significant BP Change | None | 22% 4½ hours Terminated with Prostigmine |
| E.S. | ? | M | Hypertensive Cardiovascular Disease | Yes | 135 | 2.25 | Atrial Fibrillation Ventricular 84/min | Atrial Fibrillation Ventricular 123/min | 14/100 Systoles | PVC 6/100 Systoles | BP Rise of 40 mm. Hg. | None | 31.7% Transient |
| N.S. | ? | F | Chronic Congestive Heart Failure Etiology Unknown | Yes | 60 | 1.0 | Sinus 71/min | Sinus 88/min | PNC 20/100 Systoles | PNC 0/100 Systoles | Perspiration, Lacrimation, No significant BP rise | Mild sensation of warmth. Transient itching of hands & legs | 19% 20 minutes |

Ampules containing 10 ml., 15 mg. per ml. of mephentermine sulfate were used. The drug was given undiluted intravenously by syringe, in divided doses. The range of total dosage was between 1 and 3 mg. per kilogram of body weight.

The cases were classified according to the type of arrhythmia and according to the presence of atrioventricular block.

**Results.** GROUP 1 (ECTOPIC IMPULSE FORMATION OTHER THAN ATRIAL FIBRILLATION). There were 6 patients in this group (Table 1). One patient (I.W.) was studied twice. A second patient (B) had two separate mechanisms of ectopic impulse formation, probably as a result of digitalis toxicity. Five of the patients were receiving digitalis.

The frequency of ventricular ectopic impulse formation was markedly reduced in 4 patients. (Fig. 1). This effect lasted longer than 25 minutes and was usually over in 90 minutes. In a fifth patient there was no appreciable reduction in the frequency of ventricular ectopic impulse formation. No obvious reason was apparent for this difference.

Ventricular bigeminy was present in the control electrocardiogram in 2 patients. One (A.N.) had a radial pulse of 44 per min. and an apical rate of 88 per min. Constant coupling was present. The patient was not receiving digitalis and seemed to be in remarkably good health for his age (80). At the peak effect the radial and apical pulse were the same (92 per min.) and the frequency of ectopic impulse formation was one third of control (Fig. 2). The other (I.W.) had bidirectional ventricular premature systoles. Again constant coupling was present in the control electrocardiogram. There were two foci of ectopic impulse formation with a constant alternation of the focus. The apical rate was 100 per min. with

Figure 1.

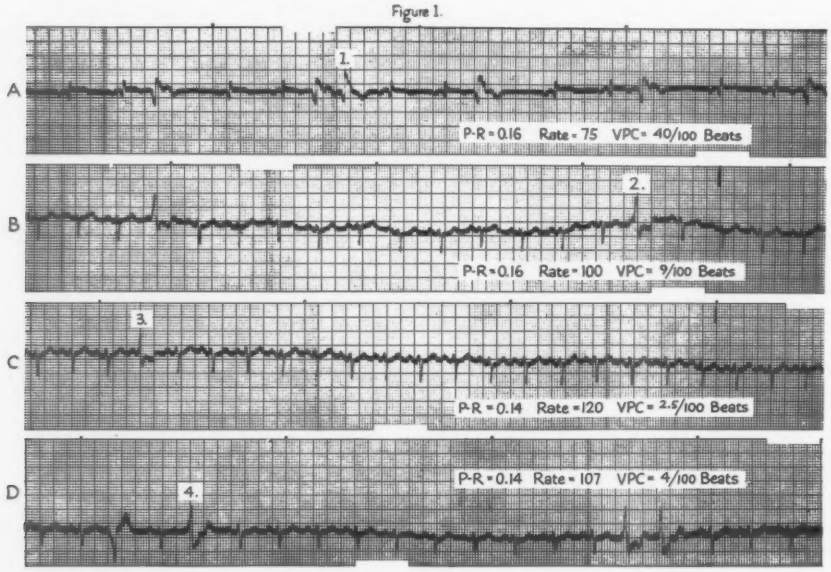
A
1.
P-R = 0.16 Rate = 75 VPC = 40/100 Beats
B
2.
P-R = 0.16 Rate = 100 VPC = 9/100 Beats
C
3.
P-R = 0.14 Rate = 120 VPC = 2.5/100 Beats
D
4.
P-R = 0.14 Rate = 107 VPC = 4/100 Beats

Fig. 2.—An 80-year-old man with hypertensive cardiovascular disease, B.P. 200/110. Control regularly. Control record (A) shows a sinus rhythm with VPC (1.). After 60 mg. mephentermine IV, VPC (2.) appeared less frequently and 10 minutes after receiving a total of 90 mg. of mephentermine, only an occasional VPC (3.) was evident with sinus tachycardia. Two hours later (D) the sinus mechanism persisted with the occasional VPC (4.).

TABLE 2.—EFFECT OF MEPHENTERMINE ON PATIENTS IN GROUP 2.

| Subject | Age | Sex | Diagnosis | Receiving Digitalis | Total Dose mg. | Dose/kg. Body Wt.—mg. | Basic Rhythm | | Ventricular Rate | | Other Effects | | Duration of Effect |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Control | Max. Effect | Control | Max. Effect | Objective | Subjective | |
| E.S. | ? | M | Hypertensive Cardiovascular Disease | Yes | 135 | 2.25 | Atrial fibrillation | Atrial fibrillation | 84/min | 123/min | BP rise of 40 mm. Hg. | None | 31.7% 60 min. |
| A.B. | 60 | M | Arteriosclerotic Heart Disease | Yes | 75 | 1.25 | Atrial fibrillation | Atrial fibrillation | 62/min | 100/min | Slight ST Segment Elevation (1½ min) | None | 38% 20 min. |
| H.W. | 52 | M | Arteriosclerotic Heart Disease | Yes | 90 | 1.8 | Atrial fibrillation | Atrial fibrillation | 96/min | 162/min | No Significant BP change | Sensation of warmth; Itching; Restlessness | 40.8% 60 min. |
| B.B. | ? | M | Arteriosclerotic Heart Disease | Yes | 195 | 3.0 | Atrial fibrillation | Atrial fibrillation | 84/min | 144/min | BP rise of 36 mm. Hg. | None | 41.66% 60 min. Average of 38% |

a radial pulse deficit. The diagnosis was active rheumatic fever, yet with very few symptoms. At the peak effect the electrocardiogram showed a sinus rhythm at a rate of 100 per min. At this time, every third systole had the appearance of a fusion complex. The QRS complex was widened (0.16 sec. rather than 0.10 sec. otherwise prestlation. The increase in the ventricular rate had a mean of 38%. The tachycardia was over within 2 hours.

GROUP 3. (ATRIOVENTRICULAR HEART BLOCK). There were 7 patients in this group (Table 3). Three had first degree A–V block, 2 second degree and 2 complete block. All the patients except one (C.H.) were receiving digitalis.

Figure 2.

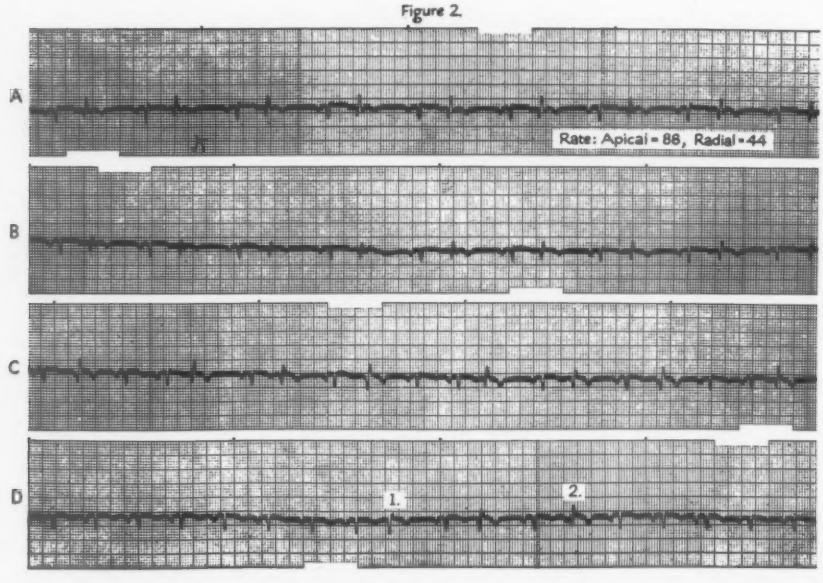


Fig. 2.—An 80-year-old man with hypertensive cardiovascular disease, B.P. 200/110. Control record (A) shows bigeminal rhythm. After 15 mg. of mephentermine (B) coupling persisted. Following 30 mg. mephentermine (C) a break in bigeminus begins. A predominant sinus rhythm with occasional extrasystoles (1) and coupling (2) is evident after 98 mg. of mephentermine.

ent), slightly different from the dominant complex, occurred early and with a preceding short PR interval.

In the sixth patient (N.S.) the premature nodal systoles were abolished with a 19% increase in rate. The increase in ventricular rate ranged from 0% to 37.5% with a mean of 16.3%.

GROUP 2. (CHRONIC ATRIAL FIBRILLATION). Each of the patients in this group had long-standing atrial fibrillation. All were receiving digitalis. There was no apparent influence on the fibril-

The patient (C.H.) had a congenital complete A–V block. In this patient (C.H.) and the other patient (S.) with complete block and A–V dissociation, the effect was to increase both the atrial and ventricular rate without reduction of the A–V block.

That there is an effect upon the atrioventricular nodal conduction was shown quite clearly in the case of the patient (B). The control electrocardiogram revealed atrial tachycardia and a second degree A–V block with 2:1

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and 1:1 conduction. The atrial rate was 187 per min. and the ventricular rate 144 per min. At the peak effect both the atrial and ventricular rates were 185 per min. The rhythm was atrial tachycardia with 1:1 conduction (Fig. 3).

In another patient (J.S.) a second degree A–V block with predominant 2:1 conduction changed to lesser degrees of A–V block with 3:2; 5:4 and 1:1 conduction. In this patient the atrial rate increased 16.6% or 20 beats per minute. The ventricular rate increased 36.6% or 33 beats per minute.

Of the 3 patients with first degree A–V block, one (I. W.) had an electrocardiographic change in the PR interval from 0.26 sec. to 0.20 sec. at the peak effect. The shortened PR interval was present 24 hours later. In this patient the heart rate did not change. The electrocardiograms of 2 other patients showed a decrease in the PR interval of 0.04 sec. The heart rate increased less than 20%.

GROUP 4 (BUNDLE BRANCH BLOCK). There was no apparent effect upon the electrocardiograms of patients with bundle branch block of organic origin other than an increased sinus rhythmicity.

NONELECTROCARDIOGRAPHIC EFFECTS. There was an inconstant elevation of the blood pressure. Five of the patients seemed to experience no subjective sensations. One patient spoke of a sense of well being. Of the remaining 8 patients, each had mildly unpleasant subjective sensations. Prominent com-

Figure 3.

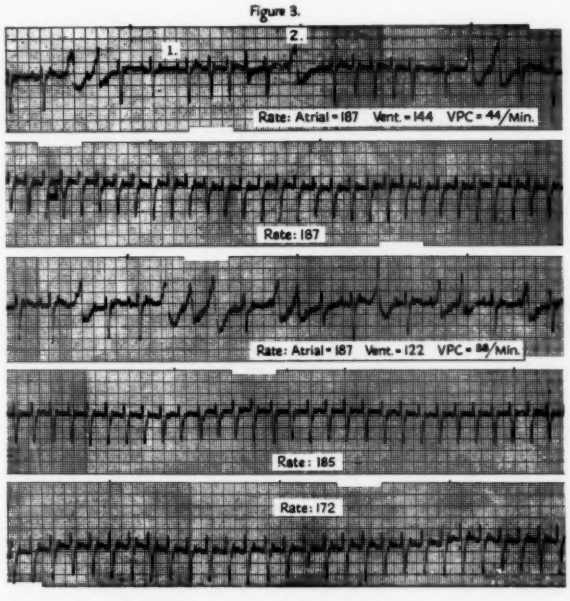


Fig. 3.—A 70-year-old man with chronic heart failure developed digitalis intoxication. Other therapy included quinidine and potassium chloride. The control record (A) shows paroxysmal atrial tachycardia with variable block (1) and VPC (2). After 15 mg. mephentermine sulfate IV (B), VPC and variable heart block disappeared. However, these abnormalities returned after 37.5 mg. mephentermine had been given (C). Following administration of a total dose of 45 mg. mephentermine (D & E) the paroxysmal tachycardia persisted and was converted with prostigmine.

TABLE 3.—EFFECT OF MEPHENTERMINE ON PATIENTS IN GROUP 3.

| *Subject* | *Age* | *Sex* | *Diagnosis* | *Receiving Digitalis* | *Total Dose mg.* | *Dose/kg. Body Wt.—mg.* | *Rhythm & Rate* | | *Degree of Block* | | *Other Effects* | | *Duration of Effect* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Control* | *Maximum* | *Control* | *Max. Effect* | *Objective* | *Subjective* | |
| N.S. | ? | F | Chronic Congestive Heart Failure Etiology Unknown | Yes | 60 | 1.0 | Sinus 71/min | Sinus 88/min 19% | First PR interval equals 0.22 sec. | None PR interval equals 0.18 sec. | No significant BP rise | Mild sensation of warmth; Transient itching of hands & legs; Prespiration | 20 min. |
| Prt. | ? | M | Coronary artery disease | Yes | 120 | 2.0 | Sinus 100/min | Sinus 120/min 16.6% | First PR interval equals 0.24 sec. | None PR interval equals 0.20 sec. | BP rise of 30 mm Hg. | Mild sensation of warmth; tingling of arms; mild substernal ache lasting 5 min. | 30 min. |
| J.S. | ? | M | Recent Myocardial Infarction | Yes | 150 | 2.0 | Sinus Atrial 100/min Ventricular 57/min | Sinus Atrial 120/min Ventricular 90/min 36.6% | Second Predominantly 2:1 conduction | Second 3:2 to sinus tachycardia | BP being supported with Noradrenalin | Sense of well being | 90 min. |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bkz. | 70 | M | Chronic congestive heart failure arteriosclerotic heart disease | Yes Toxicity? | 52.5 | 0.75 | Paroxysmal Atrial Tachycardia Atrial 187/min Ventricular 144/min | Paroxysmal Atrial Tachycardia Atrial 185/min Ventricular 155/min | Second 2:1 & 1:1 conduction | Second 1:1 conductoin | No significant BP rise | None | 4½ hours. Termination with Prostigmine |
| C.H. | 24 | F | Congenital Complete A–V Block | No | 120 | 3.0 | A–V dissociation atrial 85/min ventricular 45/min | A–V dissociation atrial 125/min ventricular 61/min | Complete | Complete | Lacrimation Incr. respiratory rate BP rise of 10 mm. Hg. | Transient generalized tingling; sense of nervousness | 30 min. |
| Sfd. | ? | F | Arteriosclerotic heart disease | Yes | 120 | 2.0 | A–V dissociation atrial 100 ventricular 31 | A–V dissociation atrial 115 ventricular 36 | Complete | Complete | Incr. respiratory rate BP rise of 20 mm. Hg. | Anxiety; transient generalized tingling; abdominal pain | 30 min. |
| I.W. | 36 | F | Active rheumatic fever | Yes | 60 | 1.0 | Sinus Apial 100/min | Sinus 100/min 0% | First PR = 0.26 sec. | None PR = 0.20 sec. | No BP change | Mild nervousness 24 hrs.; Desire to void | |

plaints included (1) apprehension or nervousness and (2) a sense of warmth and tingling or itching of the skin and (3) a desire to void. There was present a slight increase in respiratory rate, occasional nausea, perspiration and increased lacrimal gland activity.

**Discussion.** These studies show that mephentermine sulfate has an antiarrhythmic effect upon the human diseased heart. This evidence represents clinical confirmation of the results obtained in the laboratory with the experimental animal (Oppenheimer, Lynch and Ascanio[7]).

The frequency of premature ectopic impulse formation was reduced in 5 of 6 patients studied. Of these 5 cases, 2 had a significantly increased heart rate, and 3 did not. In the patient without appreciable change in frequency of ectopic impulse formation the heart rate was significantly increased.

The mechanism of ectopic impulse formation is complicated. Experimentally it has been shown that extrasystoles may originate from the edge of a necrotic zone where conduction is slow (Moe[5]). A focus of increased excitability in the myocardium may be responsible for the ectopic impulse. It is possible that with a slowing or blocking of conduction in an area of the myocardium, that automaticity remains normal, accounting for the ectopic impulse (Moe and Mendez[6]). The decreased conduction time produced by mephentermine allows the total ventricle to be refractory at one time, thus permits only one order of excitation, thereby decreasing the likelihood of ectopic impulse formation (Oppenheimer, Lynch and Barrera[8]). An increase in heart rate may be invoked as the mechanism. However, it is to be noted that the reduction in frequency of ectopic impulse formation did not correlate well with a faster heart rate.

The salutary action of mephentermine upon the heart is well seen in the 2 patients with a ventricular bigeminal rhythm and a radial pulse deficit. In the one patient (A.N.), 80 years old, who was not receiving digitalis, probably degenerative myocardial disease was responsible for a decreased velocity of conduction (prolonged conduction time) through the ventricular muscle mass (Wiggers[12]). The ectopic ventricular systole suggests abnormal excitation and the 50% radial pulse deficit suggests abnormal contraction of myocardial fractions (Wiggers[13]). The response to mephentermine was manifested by an increase in heart rate of 4 systoles per min., a reduction in the frequency of ectopic impulse formation and no pulse deficit. The myocardial action of mephentermine to increase velocity of conduction (Oppenheimer, Lynch and Barrera[8]) may correct faulty excitation and the positive inotropic action of the amine may aid contraction (Goldberg *et al.*[2], Oppenheimer, Lynch and Barrera[8], Welch *et al.*[10a]).

In the other patient (I.W.), whose diagnosis was active rheumatic fever, acute inflammatory foci may be invoked as the mechanism producing abnormal ventricular excitation.

In 4 patients with chronic atrial fibrillation and in one patient with atrial tachycardia, no salutary effect upon the cardiac action was observed. Therefore, we were unable to confirm, clinically, the thesis that the excitatory gap of a circus movement may be closed by increasing the velocity of conduction. Dog experiments were in acute normal hearts, however, and these observations were in chronic cases probably with organic heart disease.

In another patient (B.) mephenter-

mine, in the doses used, had no recognizable effect upon the atrial tachycardia. However, the second degree atrioventricular block was abolished. This response is consistent with the action of mephentermine on the atrioventricular node and bundle as delineated in the experimental dog (Stewart *et al.*[10]).

Recently, there has been an apparent increase in the number of patients with atrial tachycardia and second degree atrioventricular block. It is felt that this may represent more complete diuretic control of patients receiving digitalis (Soloff[9]). We have not pursued the use of mephentermine in this arrhythmia. At the present it is our feeling that a supraventricular tachycardia with atrioventricular block represents a contraindication to the use of mephentermine.

The action of single doses of mephentermine on the conductile tissue is to reduce the refractory period and to increased the velocity of conduction (Stewart *et al.*[10]). In 3 patients with first degree atrioventricular block the conduction time was shortened to within normal limits. One patient (B.) with second degree atrioventricular block was discussed previously. In another patient the block was reduced indicating a reduction of the refractory period. In 2 patients with complete atrioventricular dissociation the electrocardiographic findings indicate an increase in both atrial and ventricular rhythmicity. Lenthening of A–V conduction time observed in dogs with second doses or prolonged administration was not seen here[1a].

The anti-arrhythmic actions of mephentermine suggest that this drug may be of greater value during anesthesia than generally recognized (Lynch, Webber and Oppenheimer[3]). The antifibrillatory action and the action upon the conduction system may be of value in the field of intracardiac surgery with the extracorporeal pump-oxygenator and the use of therapeutic cardiac arrest. Its usefulness against ventricular fibrillation in hypothermia is already established (Covino[1]).

In patients with chronic congestive heart failure, theoretically, mephentermine as an oral preparation may find use as an adjuvant to digitalis, because of the direct action upon the heart muscle to increase the force of contraction and to facilitate conduction.

**Summary.** Mephentermine sulfate has an anti-arrhythmic effect upon the human diseased heart. This salutary action was especially noted in patients with ventricular ectopic systoles.

The mechanism of action is a decrease in the refractory period, an increase in the velocity of conduction and a positive inotropic effect.

In doses up to 3 mg. per kilogram administered intravenously there was no significant electrocardiographic effect in patients with chronic atrial fibrillation.

Effective reduction in functional atrioventricular block was produced. Supraventricular tachycardia with second degree atrioventricular block represents a contraindication to the use of mephentermine.

The antifibrillatory properties of mephentermine may be of value in open heart surgery with therapeutic cardiac arrest.

Theoretically, an oral preparation of mephentermine may find use as an adjuvant to digitalis in patients with chronic congestive heart failure.

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## SUMMARIO IN INTERLINGUA

### Mephentermina E Le Arrhythmias

Sulfato de mephentermina exerce un effecto anti-arrhythmic super le morbide corde human. Su action salutatori esseva notate specialmente in patientes con ectopic systoles ventricular.

Le mechanismo del action in question se manifesta in un reduction del periodo de refractorietate, in un augmento del velocitate de conduction, e in un positive effecto inotropic.

Post doses de usque a 3 mg per kg, administrate per via intravenose, nulle significative effecto electrocardiographic esseva constatate in patientes con chronic fibrillation atrial.

Un reduction effective del bloco atrio-ventricular functional esseva producite. Tachycardia supraventricular con bloco atrio-ventricular del secunde grado es un contraindication al uso de mephentermina.

Le virtutes antifibrillatori de mephentermina es possibilemente de valor in cardiochirurgia aperte in casos de arresto cardiac therapeutic.

Theoricamente, un preparato oral de mephentermina pote trovar uso como adjuvante a digitalis in patientes con chronic insufficientia congestive del corde.

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# CHEMICAL CONSTITUENTS OF PINE POLLEN AND THEIR POSSIBLE RELATIONSHIP TO SARCOIDOSIS*

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Epidemiological studies of a large series of patients with sarcoidosis revealed an interesting geographic distribution of the disease in the Eastern United States (Michael *et al.*[4], Cummings *et al.*[3]). The concentration of cases correlated well with the forest distribution in this country (Fig. 1). Using this finding as a clue to etiology of the disease, a systematic study of various forest products (chemical and biological) was undertaken. During these studies a series of interesting observations have been noted which are the basis for this report.

Nethercott and Strawbridge[5] claim

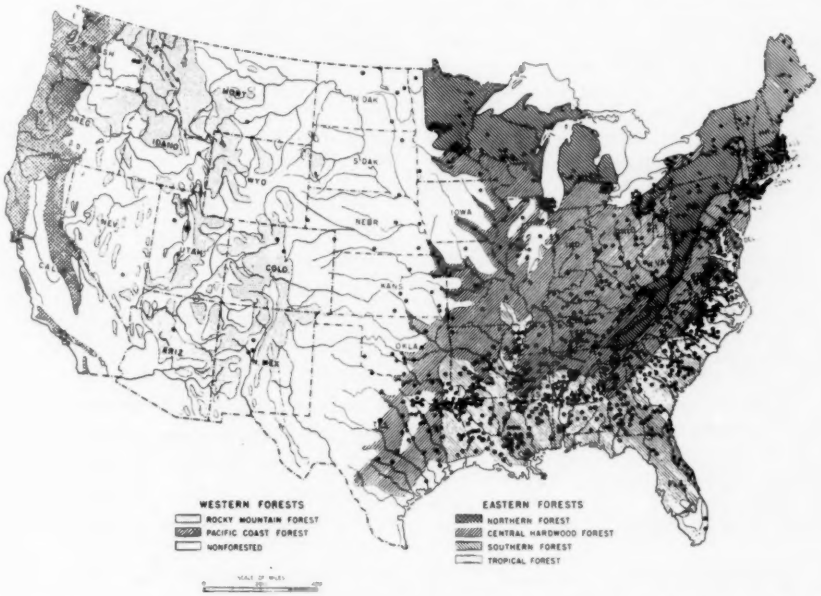


Fig. 1.—Place of birth of veterans with sarcoidosis superimposed upon the forest distribution in the United States.

*Presented at the 50th Annual Meeting of the American Society of Clinical Investigation, Atlantic City, N.J., May 5, 1958.

to have found mycolic and alpha epsilon diamino pimelic acids in lung and lymph node tissues of patients with sarcoidosis. Since these acids occur in tubercle bacilli and are not found in normal mammalian tissues, they reasoned that sarcoidosis was a manifestation of tuberculosis.

Since we have found that the pine pollen has acid-fast staining qualities similar to those of the tubercle bacillus, we decided to determine whether this was due to the presence of lipids comparable to those described in the tubercle bacillus (Stodola, Lesuk and Anderson[8]). It also seemed desirable to search for presence of alpha epsilon diamino pimelic acid shown to exist in the tubercle bacillus by Asselineau, Choucroun and Lederer[2].

**Methods.** Several varieties of pine pollen were examined microscopically for morphological and tinctorial properties. Microscopic examination of wet-mount preparations of the pollens revealed a rather large round central cell body with bilateral wing-like air bladder appendages. Micrometer measurements of loblolly, slash, longleaf, shortleaf, california, white and black pine pollen, ranged from 45 to 50 micra in diameter.*

In attempting to suspend pine pollen in aqueous media, it became apparent that they were extremely resistant to wetting action, suggesting a hydrophobic coating on the cell wall. Therefore attempts to demonstrate the presence of lipoidal materials by staining were undertaken. The standard Ziehl-Neelsen method of staining used for the tubercle bacillus revealed that the pollen cells were markedly acid-fast, whereas the air bladders did not demonstrate acid fast characteristics (Fig. 2). In addition, pollens from ginkgo and golden larch (two other gymnosperms) demonstrated the same staining properties. Several varieties of tree pollens other than gymnosperms (oak, mulberry, sycamore, plantain, maple and elm) failed to show acid fast properties.†

The chemical methods of Anderson[1] were used to isolate a so-called "purified wax" fraction from the white pine pollen and from heat-killed human type (H 37 Rv) tubercle bacilli. Dry organisms were extracted four days with a 50–50 alcohol-ether mixture and an additional 4 days with chloroform. The chloroform extract was evaporated to dryness and the residue dissolved in ethyl ether. An equal volume of cold methanol was added to the other solution and a fine white precipitate formed overnight at 5° C. The insoluble material was redissolved in ethyl ether and reprecipitated with cold methanol. The resultant precipitates were dried *in vacuo* over $P_2O_5$ and then finally ground in a mortar and pestle with potassium bromide. These mixtures were pressed into pellets for infrared spectroscopy. Analysis was performed with a Model 21 Perkin-Elmer recording infrared spectrometer with sodium chloride optics.‡

Dry pine pollen (white and california) and heat-killed tubercle bacilli, were subjected to 6N-hydrochloric acid hydrolysis for 8 hours at 125° C., and treated according to the method reported by Nethercott and Strawbridge[5] for the identification of alpha epsilon diamino pimelic acid in sarcoid tissues. The resulting hydrolysates, and a 20 mcg. sample of pure D.A.P.§ were analyzed by the paper chromatographic technique of Rhuland *et al.*[6] using a methanol, water, HCl, pyridine solvent system.

**Results.** The infra-red spectra of pine pollen "purified wax" and tubercle bacillus "wax" are presented in Fig. 3. The material from pine pollen and from the tubercle bacilli are very similar. However, they differ somewhat from the material isolated from lesions of sarcoidosis (Nethercott and Strawbridge[5]). Both the pine pollen and the tubercle bacillus "wax" spectra are similar to a fraction isolated from the tubercle bacillus by Smith *et al.*[7] and

*Pine pollens were kindly supplied by Drs. J. A. Hall, Forest Products Lab., U.S.D.A., Madison, Wis., and E. L. Patton, Naval Stores Station, U.S.D.A., Olustee, Fla.

†Tree pollens were kindly supplied by Dr. Harry S. Bernton, Washington, D.C.

‡Infra-red spectroscopy performed by Rita A. McCallum, Nat. Cancer Inst., Bethesda, Maryland.

§Sample of pure D.A.P. procured from the Mann Research Laboratories, New York, N.Y.

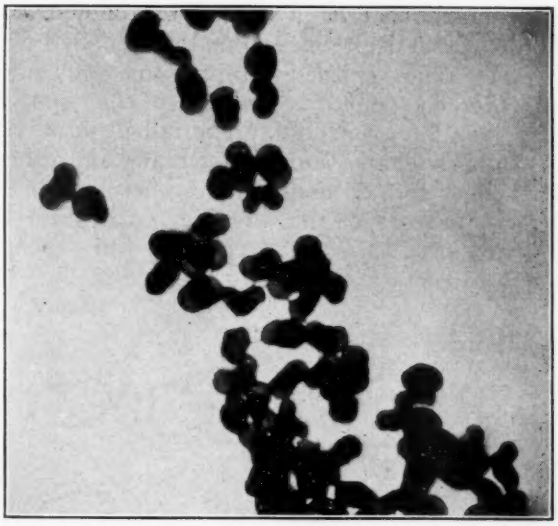

Fig. 2.—Photomicrograph of white pine pollen stained by the Ziehl-Neelsen technique.

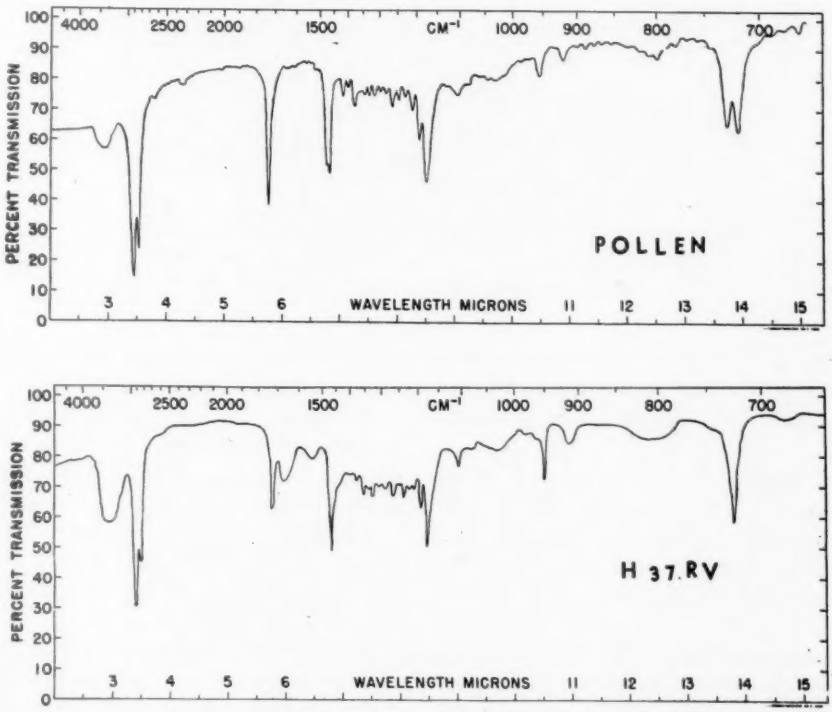


Fig. 3.—Infra-red spectra of "purified wax" fractions isolated from white pine pollen and human type tubercle bacilli.

designated as Compound C. This compound has been characterized as a diglyceride, composed of at least one long chain fatty acid. The reported melting point for this compound is 59.5 to 60.5°. The melting points of our pine pollen lipid fraction and tubercle bacillus fraction were 53 and 52° C. respectively. A mixture of the two compounds melted at 52° C.

the same Rf value as D.A.P., but giving a slightly different color reaction, was found in the hydrolysate of the california pine pollen. The olive-green ninhydrin color spots on the chromatogram faded to a yellow-green color on standing and exhibited a faint pink fluorescence under ultraviolet light. This chromatogram is similar to that reported for D.A.P. by Work[9],

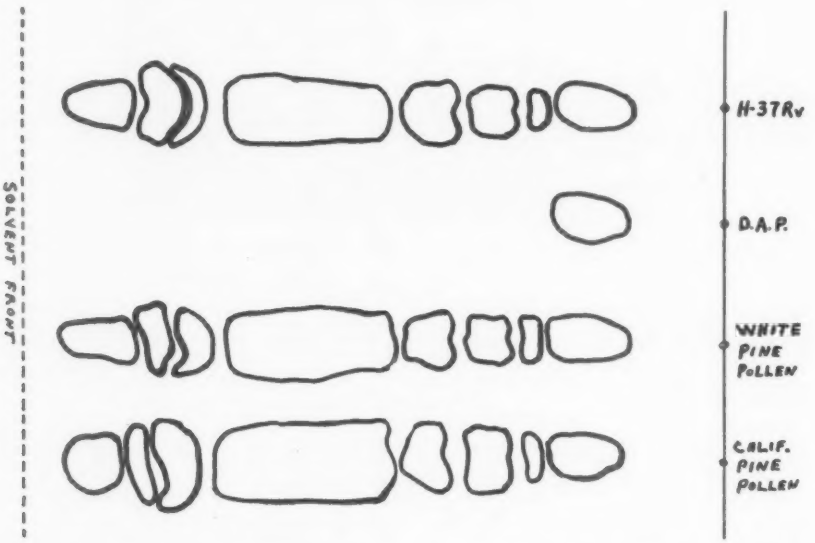


Fig. 4.—Paper chromatogram (tracing) of acid hydrolysates from H37 Rv tubercle bacilli, white and California pine pollen, and 20 mcg. sample of D.A.P.

Mycolic acid could not be demonstrated in pine pollen lipids by chromatographic and spectrographic techniques.*

The paper chromatogram of white pine and california pine pollen, tubercle bacillus and pure D.A.P. acid hydrolysates is presented in Fig. 4. An amino acid having an Rf value of 0.23 and giving the olive-green ninhydrin color reaction characteristic of the control D.A.P. was present in the hydrolysate of the white pine pollen and the tubercle bacillus. An amino acid with

Asselineau, Choucroun and Lederer[2], and Nethercott and Strawbridge[5].

Using the methods of Anderson[1] for fractionation of the tubercle bacillus lipids, we have also found that the pine pollen contains phospholipid, chloroform soluble waxes, alcohol-ether soluble lipids and firmly bound lipids, similar to those described for the tubercle bacillus.

Suspensions of pine pollen in paraffin oil injected intracutaneously in guinea pigs rendered hypersensitive to tuberculin with Freund's adjuvant,

*Kindly performed by Dr. Edgar Lederer, Paris, France.

evoked indurated nodules in 4 to 5 days. These nodules persisted for 10 days and then disappeared. Five months later the animals were sacrificed and revealed a small round lesion at the site of the injection as well as enlargement of the regional lymph nodes. Histological studies of these lesions in the subcutaneous tissues and of an area of mediastinal tissue demonstrate the presence of epithelioid cell

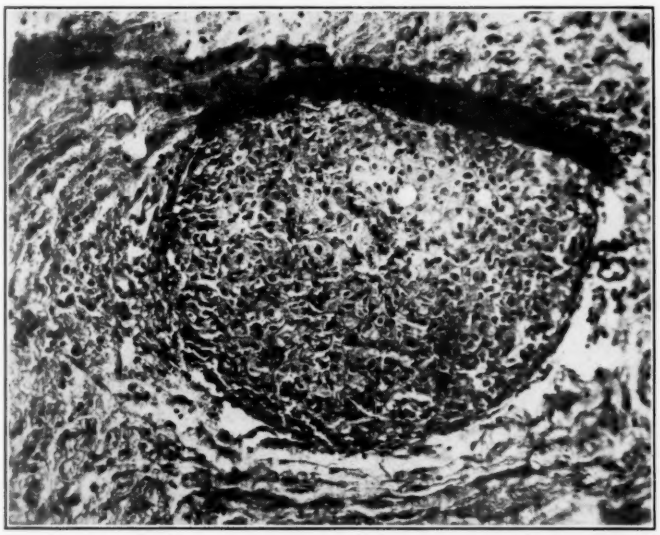

Fig. 5.—Photomicrograph (150 ×) of "hard tubercle" formation in subcutaneous tissue of normal guinea pig 5 months following the injection of pine pollen suspended in paraffin oil.

granulomata similar to the "hard tubercle" (Figs 5 and 6). These tissues do not reveal acid-fast bacilli when stained with carbol fuchsin. Therefore, we do not believe that the lesions were produced by the Freund's adjuvant. Stained preparations of tissues from animals receiving Freund's adjuvant alone did reveal acid-fast bacilli. Pine pollen suspensions injected into normal guinea pigs revealed local lesions with no extension to other organs. Similar lesions were noted when the pollen was ground in a ball mill and was injected as a saline suspension. Although these preliminary experiments have not succeeded in producing widespread systemic involvement, we believe further experiments involving longer incubation periods are justified.

From the alcohol-ether soluble fraction of pine pollen, an acetone insoluble phosphatide was obtained which when injected intracutaneously in normal guinea pigs, induced an epithelioid cell reaction in 5 to 7 days. Serial biopsy of the lesions revealed the presence of epithelioid and numerous giant cells in 21 days. Also, giant cells were demonstrated in splenic tissues of rabbits and guinea pigs, 21 days following intraperitoneal or subcutaneous injections of this pine pollen phosphatide (Fig. 7).

**Discussion.** The finding of an acid-fast lipid fraction similar to a lipid fraction of the tubercle bacillus and D.A.P. in pine pollen obtained from the geographic area of sarcoidosis leads to interesting speculation. It does not support the implication that the

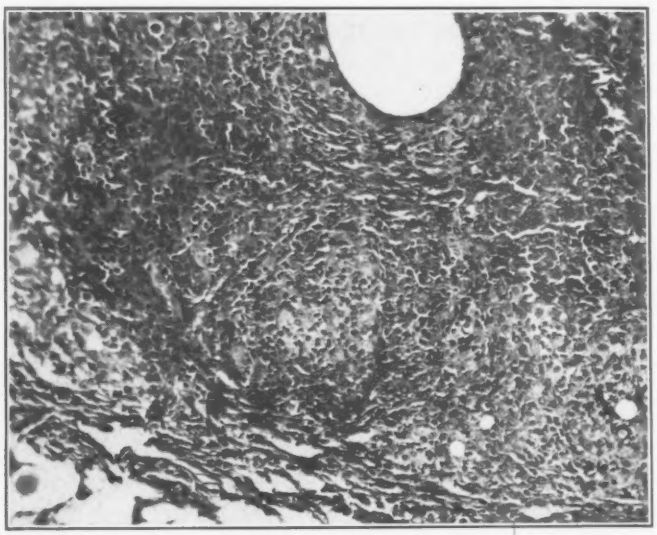

Fig. 6.—Photomicrograph (150 ×) of epithelioid cell reaction in mediastinal tissues of guinea pig 5 months following the injection of pine pollen suspended in paraffin oil.

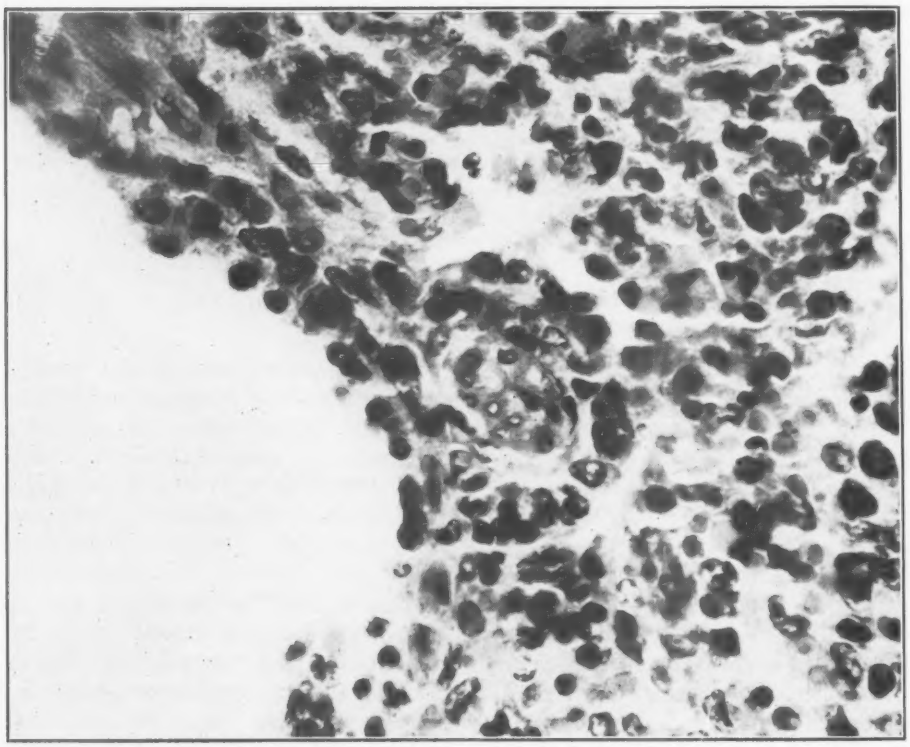

Fig. 7.—Photomicrograph (600 ×) of giant cell in the splenic tissues of guinea pig 21 days following the injection (S.C.) of Pine pollen phosphatide fraction suspended in saline.

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presence of these constituents in sarcoid lesions necessarily points to the tuberculous etiology of sarcoidosis suggested by Nethercott and Strawbridge[5]. Indeed we cannot agree that these workers demonstrated mycolic acid in lesions from sarcoidosis but rather we believe they found a less specific material composed of a triglyceride of fatty acids. We believe they have good evidence that D.A.P. exists in sarcoid lesions. It is not inconceivable, however, that the presence of an acid-fast lipid and D.A.P. in mammalian tissues might be the result of exposure of the susceptible host to pine pollen, or indeed it may be learned that similar compounds exist in a wide variety of biological materials.

It is suggested that pine pollen may be added to the list of chemical and biological agents capable of inducing epitholioid cell granuloma. We offer this as a suspect which fits the epidemiologic pattern of sarcoidosis, although proof is still lacking to establish it as a cause of the clinical disease or syndrome.

**Summary.** 1. Pine pollen has been demonstrated to have acid-fast staining characteristics similar to the tubercle bacillus.

2. An acid-fast lipid and an amino acid having the characteristics of alpha epsilon diamino pimelic acid have been found as constituents of the pine pollen.

3. Pine pollen is capable of evoking epithelioid cell granulomata in normal animals. More diffuse lesions are found in tuberculin-hypersensitive animals and in normal animals injected with chemical fractions derived from pine pollen.

ACKNOWLEDGMENTS: We are grateful to Dr. Max Michael who first suggested that sarcoidosis might be related to an environmental factor associated with the rural distribution of the disease, and to Dr. Samuel P. Martin who called to our attention the fact that pine pollen may occasionally be found in sputum during the spring and summer months. Dr. D. W. Smith kindly reviewed our infra-red spectrograms and pointed out the similarity of the pollen "purified wax" to a diglyceride isolated from the tubercle bacillus.

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*(Interlingua on page 329)*

# SYSTEMIC LUPUS ERYTHEMATOSUS: A CLINICAL REPORT OF 47 CASES WITH PATHOLOGIC FINDINGS IN 18

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Lupus erythematosus, once thought to be a medical curiosity, has been diagnosed with increasing frequency since the description of the "LE" phenomenon by Hargraves[10] in 1948; it is now seen on a general medical service as often as such diseases as pernicious anemia (Dubois[5]) and acute glomerulonephritis.

Lupus erythematosus in its discoid form was first described by Biett[2] in 1833; in 1872, Kaposi[15] called attention to the systemic manifestations of the disease. Libman and Sacks[19] described the nonbacterial verrucous endocarditis seen in systemic lupus erythematosus (hereafter called SLE) in 1924. The pathological changes in 23 patients were excellently detailed by Baehr, Klemperer and Schifrin[1].

It is only since Hargraves discovery, however, that it has been possible to compile large clinical groups of patients with SLE. Studies on such groups have been made by Dubois[5], Harvey[11], Jessar[14] and Haserick[12]. It is our purpose to present observations on 47 patients from the John Gaston Hospital with this diagnosis and to compare our data with that from other large series.

INCIDENCE. Forty (85%) of our patients were Negroid; 7 were Caucasian. The racial incidence corresponds to the percentage of each group admitted to this hospital (89 and 11% respectively), a fact previously noted by Dubois[5] and Harvey[11]. Three (7%) were males; 44 (93%) were females. The incidence of SLE in females of this series falls between the extremes reported by Harvey (78%) and Jessar (98%). Table 1 compares total admissions and medical admissions for 7 years with total admissions for SLE in the same years. It can be seen that SLE accounts for .33% of medical and .043% of total hospital admissions.

TABLE 1.—A COMPARISON OF TOTAL ADMISSIONS WITH ADMISSIONS DUE TO LE

| *Year* | *1949* | *1950* | *1951* | *1952* | *1953* | *1954* | *1955* |
|---|---|---|---|---|---|---|---|
| Total admissions | 17,808 | 18,587 | 19,056 | 20,608 | 21,339 | 21,961 | 21,642 |
| Medicine admissions | 2,535 | 2,464 | 2,867 | 2,718 | 2,669 | 2,382 | 2,772 |
| Admissions for LE | 4 | 11 | 10 | 15 | 9 | 7 | 4 |



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The age of onset, by history of first symptom due to SLE, is depicted graphically in Fig. 1. From this graph it is apparent that the most frequent age of onset was the third decade of life. The oldest patient was 58 years old at the appearance of initial symptoms; the youngest was 6 years old.

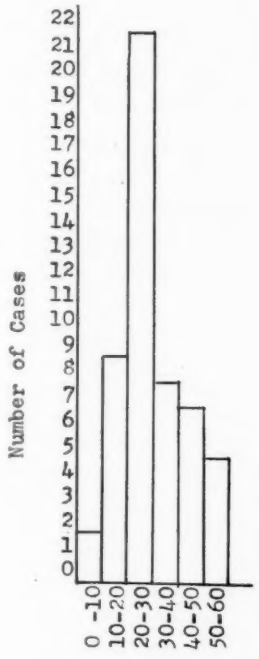


Fig. 1

SYMPTOMS AND PHYSICAL SIGNS. Arthritis and arthralgia were by far the most common initial manifestations of SLE in our patients. Sixteen of our 47 patients presented first with arthritis or arthralgia alone while an additional 6 patients presented with arthritis and, concurrently, another symptom of SLE. Other common presenting symptoms were skin eruption, malaise, fatigue, weakness and fever. An occasional patient presented with purpura, gastrointestinal symptoms, cardiovascular symptoms, weight loss, positive serology or neurological symptoms. Such an initial manifestation may precede the full-blown picture of SLE by months or years. The actual frequency of initial manifestations may be seen in Table 2. From the Table, which includes comparable data from 2 other series, it can be seen that, in essence, the figures correspond. The time interval between the first symptom referable to SLE and the first hospital admission varied from 2 days to 20 years in this series.

An interesting case with an unusual mode of onset is presented below; other unusual syndromes in SLE have been well described by Meacham and Weisberger[20].

**Case Reports.** CASE 1. E.S., a 33-year-old colored woman was admitted to the John Gaston Hospital on January 22, 1955, with the chief complaint of vaginal bleeding of

TABLE 2.—INITIAL SYMPTOM

| | *This Series* | | *Harvey 105 Pts.* | *Dubois 62 Pts.* | *Total 214 Pts.* |
|---|---|---|---|---|---|
| | *Patients* | *%* | *%* | *%* | *%* |
| Arthralgia | 18 | 38 | 47 | 34 | 42 |
| Skin eruption | 14 | 30 | 20 | 27 | 24 |
| Fever | 5 | 11 | 23 | 1.6 | 14 |
| Malaise, fatigue | 3 | 6 | 17 | — | 14* |
| Gastrointestinal | 3 | 6 | 5.7 | 1.6 | 4.6 |
| Neurological | 2 | 4 | 1 | — | 2* |
| Purpura | 1 | 2 | 2 | — | 2* |
| Weight loss | 1 | 2 | 7 | 1.6 | 4.2 |

If 2 complaints presented simultaneously, the more diagnostic was recorded.
*152 patients.

3-weeks' duration. Previous menstrual periods had been normal and at regular intervals of 28 days. Her last menstrual period was on December 20, 1954. The past history revealed the presence of grand mal epilepsy since the age of 9 years. She was followed in the Outpatient Department of the John Gaston Hospital, and her seizures were controlled by Dilantin, 0.4 gm. daily. Physical examination revealed no significant abnormalities.

*Laboratory.* The hematocrit was 12%. The total white cell count ranged between 2600 and 7600 per c.mm. Thrombocytes were 4 per 100 oil immersion fields (normal 300 to 1000), and the bleeding time was 20 minutes. The tourniquet test was strongly positive, and two LE cell preparations were negative. The total protein was 7.9 gm.% with albumin 3.4 gm.%, and globulin 4.5 gm.%. Two bone marrow aspirations showed an adequate number of nonplatelet forming megakaryocytes. Since no rise in thrombocytes occurred and the patient continued to bleed, on May 5, 1955, a splenectomy was performed. Pathological examination revealed a spleen weighing 238 gm., with lymphocytic and reticuloendothelial hyperplasia; the vessels were of normal appearance. The result of the splenectomy was not impressive. Just following surgery the thrombocyte count rose to 224 per 100 oil immersion fields but fell after 10 days to presplenectomy levels. Bleeding was controlled by oral corticosteroid administration, and Dilantin was replaced by phenobarbital, 0.4 gm. daily, since it was thought that Dilantin might possibly be responsible for the thrombocytopenia. The platelet count returned to normal in October, 1956. The patient was readmitted on April 6, 1956, with the chief complaint of fever, lethargy and joint swelling of 3-weeks' duration. Physical examination revealed the blood pressure to be 115/80, the temperature 100.4°, and respirations 24 per minute. The right wrist and knee were red, hot and tender and contained an effusion. A generalized nontender lymphadenopathy was noticed. A chest roentgenogram showed bilateral minimal pleural effusion. Laboratory studies revealed the following: The hematocrit was 28%. The total white count ranged between 3300 and 10,800 with a normal differential. Platelets were normal in number. Urinalysis revealed 2+ protein, 3 to 4 leukocytes and red blood cells, and a few granular casts per high power field. Total proteins were 9.5 gm.% with 2.2 gm. albumin and 7.3 gm. globulin. The sedimentation rate was 61 mm. per hour and 3 LE cell preparations were positive. During the hospital course a pericardial friction rub appeared, and the electrocardiogram showed serial ST–T wave changes compatible with pericarditis. The patient was given corticosteroids and showed an excellent response with complete disappearance of symptoms. She has been admitted twice since that time; on both occasions fever, joint symptoms and bilateral pleurisy recurred when the corticosteroids were discontinued. Administration of corticosteroid therapy relieved symptoms. The patient is at present (April, 1957) asymptomatic and is maintained on 30 mg. hydrocortisone daily.

CLINICAL CHANGES IN *SLE.* In Table 3 the changes observed during the course of SLE in our patients are enumerated and compared with 3 other series (Dubois[5], Harvey *et al.*[11] and Jessar, Lamont-Havers and Ragan[14]). General manifestations were the most ubiquitous of all changes. Fever was present at some time in all 47 patients; weight loss was present in 40 patients. Forty-one patients had synovitis; 28 of these exhibited objective evidence of joint inflammation, while 13 patients complained of aching joints without objective findings. Skeletal deformities as manifested clinically by permanent limitation of joint motion or by roentgenographic abnormalities were infrequent. Seven of the 28 patients with objective arthritis sustained deformities which were indistinguishable from those of rheumatoid arthritis, on clinical as well as on radiological grounds. The proximal interphalangeal, metacarpophalangeal, and knee joints were most frequently affected; ankles, elbows and wrists were less frequently involved.

A total of 41 patients had skin lesions of one or more types. The classical butterfly eruption occurred in 29 patients; in most of these, an eruption occurred in another location also. Twenty-eight (59%) of our patients showed areas of hyperpigmentation. In 8 patients, the only skin manifestations present during the course of the

TABLE 3.—INCIDENCE OF SIGNS AND SYMPTOMS DUE TO SLE

| | *No. of Patients* | *% of Patients* | *Harvey 105 Pts. %* | *Dubois 62 Pts. %* | *Jessar 44 Pts. %* | *Total 258 Pts. %* |
|---|---|---|---|---|---|---|
| General | | | | | | |
| Fever | 47 | 100 | 86 | 97 | 95 | 93 |
| Weight loss | 40 | 85 | 71 | 82 | 100 | 81 |
| Joints | | | | | | |
| Total | 41 | 87 | 90 | 90 | 77 | 88 |
| Arthralgia alone | 13 | 28 | — | — | — | — |
| Arthritis | 28 | 59 | — | — | — | — |
| Deformities | 7 | 15 | 27 | 30 | — | 25† |
| Skin and mucous membranes | | | | | | |
| Total | 41 | 87 | 85 | 84 | 68 | 82 |
| Butterfly | 29 | 62 | 39 | 69 | — | 53† |
| Nonspecific generalized | 3 | 6 | — | — | — | — |
| Hyperpigmentations | 28 | 59 | 12 | 16 | — | 24† |
| Photosensitivity | 7 | 15 | 11 | 40 | — | 21† |
| Purpura | 6 | 13 | 9 | — | — | 10.5‡ |
| Alopecia | 10 | 21 | 3 | 51 | — | 21† |
| Mucous membranes | 12 | 26 | 14 | 11 | 18 | 16.3 |
| Reticuloendothelial system | | | | | | |
| Total | 46 | 98 | — | — | — | |
| General lymphadenopathy | 32 | 68 | 34 | 42 | 37 | 42.6 |
| Local lymphadenopathy | 6 | 13 | 24 | — | — | 20‡ |
| Hepatomegaly | 20 | 43 | 32 | 34 | 29 | 34 |
| Splenomegaly | 9 | 19 | 15 | 8 | 27 | 16.3 |
| Serous membranes | | | | | | |
| Total | 37 | 79 | — | — | — | — |
| Pleurisy | 23 | 49 | 56 | 60 | 20 | 49.6 |
| Pleurisy with effusion | 14 | 30 | 16 | 55 | 39 | 31.8 |
| Pericarditis | 11 | 23 | 45 | 44 | 23 | 36.8 |
| Ascites | 9 | 19 | — | 24 | — | 22§ |
| Lungs | | | | | | |
| Pneumonitis | 24 | 51 | 45 | — | 20 | 41‖ |
| Eyes | | | | | | |
| Retinal lesions | 21 | 45 | 30 | 32 | 20 | 31.8 |
| Cardiovascular system | | | | | | |
| Total | 39 | 83 | 52 | — | 70 | 64 |
| Cardiomegaly | 24 | 51 | 15 | — | 34 | 28‖ |
| Heart murmurs | 27 | 57 | 44 | 42 | 55 | 47.8 |
| Hypertension* | 10 | 21 | 14 | — | 18 | 17‖ |
| Failure | 19 | 40 | 8 | — | — | 18‡ |
| EKG abnormality | 23 | 49 | 5 | 37 | — | 24† |
| Gastrointestinal tract | | | | | | |
| Total | 26 | 55 | — | — | — | — |
| Abdominal pain | 17 | 36 | 10 | 37 | 22 | 23.2 |
| Vomiting | 15 | 32 | 11 | 40 | — | 24.4† |
| Nausea | 14 | 30 | 14 | 36 | 18 | 22.8 |
| Diarrhea | 5 | 11 | 8 | 21 | — | 11.6† |
| Melena | 4 | 8 | 5 | — | — | 6‡ |
| Hematemesis | 4 | 8 | — | — | — | — |
| Dysphagia | 4 | 8 | 6 | — | — | 6.6‡ |
| Central Nervous System | | | | | | |
| Total | 17 | 36 | 38 | 60 | 18 | 39.5 |
| Convulsions alone | 7 | 15 | 17 | 31 | 7 | 18.2 |
| Psychiatric impairment | 6 | 13 | 19 | 29 | 9 | 17.4 |
| Chorea and convulsions | 1 | 2 | — | — | — | — |
| Facial weakness and hypesthesia | 1 | 2 | — | — | — | — |
| Nystagmus and convulsions | 1 | 2 | — | — | — | — |
| Transient hemiparesis | 1 | 2 | — | — | — | — |
| Hemiplegia | — | — | 2 | — | 2 | 1.5‖ |

*Diastolic blood pressure of more than 100 mm. Hg before the appearance of uremia.
‡152 patients §109 patients
†214 patients ‖196 patients

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disease were scattered areas of hyperpigmentation. This is a much higher incidence of this change than has been reported in other series. It is thought that this may be related to the fact that our patients are predominantly Negroes. It is well known that dark skins respond to any trauma with marked hyperpigmentation and also depigmentation. The low incidence of photosensitivity in this series may also be accounted for by the pre-existing darkness of the patients' skins. Only a generalized maculopapular rash presented in 3 patients. Purpura, a rare finding in SLE, was present as the solitary skin lesion in only one patient. One-fourth of the patients having skin lesions in this series had alopecia secondary to scalp lesions. Prior to Hargraves's description of the LE cell, no case was clinically diagnosed in this series in the absence of skin changes.

Involvement of the reticuloendothelial system was very common. Generalized lymphadenopathy was present in 32 patients; localized lymphadenopathy in 6 patients. No explanation can be offered for the relatively high incidence of lymphadenopathy in this series. The liver was palpably enlarged in 20 of our patients; the spleen in 9.

The classical frequent occurrence of serous membrane involvement was confirmed by the demonstration of pleurisy in 23 patients, ascites in 9, and pericarditis in 11. The clinical diagnosis of pericarditis was made either by asculation of a friction rub or by aspiration of a pericardial effusion; it is interesting to note that microscopic examination of the pericardial fluid in one patient revealed typical LE cells.

Pneumonitis was present at some time in 24 of our patients; this had the physical and radiological characteristics of a bronchopneumonia. An occasional case showed plate atelectasis. A number of these pulmonary infiltrates resolved on steroid therapy alone without the use of antibiotics. The definite diagnosis of lupus pneumonia (noninfectious) could be verified only at necropsy in these patients.

Fundoscopic lesions, as evidenced by exudates, hemorrhages, or cytoid bodies, were observed in 21 patients.

Involvement of the cardiovascular system was marked by cardiomegaly in approximately one-half of our cases. Heart murmurs were present frequently in the apical or pulmonic region but had no distinguishing characteristics. Ten of our patients manifested a diastolic hypertension of 100 mm. Hg or more. Clinical evidence of cardiac decompensation occurred at some time during the course of the disease in 19 patients. It is our opinion that the relatively high incidence of cardiovascular complications in this series may be related to the known high incidence of hypertensive cardiovascular disease in Negro people in the United States (Friedberg[7] and White[24]); in other words, the hypertension may not be related to the SLE. Of the 9 patients in this series with clinical ascites, 7 also had definite cardiac failure. Cardiac failure was also present in 12 of the 20 patients with hepatomegaly and might account at least in part for these signs. Electrocardiograms were performed on 39 patients. Fourteen of these were normal or showed only sinus tachycardia. Ten electrocardiograms revealed low voltage; of these 8 were on patients who either clinically or at necropsy had a pericardial effusion. Only 4 electrocardiograms showed changes compatible with pericarditis. An atrioventricular conduction defect (first degree atrioventricular block) was found on the tracings of 4 patients; 2 of these were on digitalis therapy at the time of the tracing and 2 were not. This incidence of conduction defect corresponds with the incidence found

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in Harvey's larger series[11]. Eight patients had nonspecific ST–T wave changes or P-wave abnormalities. Left ventricular strain pattern was present in 3 patients.

One or more gastrointestinal symptoms occurred in 27 of this series; of these, the most prominent were pain (17 patients), vomiting (15) and nausea (14). Usually some explanation such as hepatomegaly or bleeding diathesis could be found to account for the gastrointestinal symptoms, but this was not always the case.

CASE 2 .C.P., a 22-year-old colored woman, was seen for the first time in March, 1956. At that time the diagnosis of SLE was made. The diagnosis was based on typical butterfly lesions in the face, polyarthritis involving hands, ankles and knees, lymphadenopathy, and moderate splenic enlargement. The laboratory tests showed a hematocrit of 32%, leukocyte counts that ranged between 2900 and 4800 per c.mm. and a positive LE preparation. Total serum proteins were 8.3 gm. with an albumin of 4.3 gm. and a globulin of 4.0 gm. The patient was treated on the outside on 15 mg. prednisone daily. On August 26, 1956, the patient was admitted to the Surgery Service with the chief complaints of diarrhea and vomiting of one day's duration. She was complaining of severe intermittent colicky abdominal pain. The physical examination showed a well-developed woman in acute distress. The blood pressure was 150/110, pulse 84, respirations 24 and temperature 99.6°. The abdomen was distended. There was marked periumbilical rebound tenderness. No bowel sounds were heard. Laboratory studies revealed a hematocrit of 36%, the leukocytes numbered 5800, with a differential count of 20% bands, 58% segmented neutrophils, 13% lymphocytes and 4% monocytes. The erythrocyte sedimentation rate was 45 mm. fall per hour. Urinalysis revealed 4+ protein, red blood cells too numerous to count, leukocytes 3 to 5 and a few coarse granular casts per high power field. Serum amylase of 75 Somogyi units was recorded. Nonprotein nitrogen was 30 mg. per 100 ml. Total serum proteins were 5.7 gm., with 2.9 gm. albumin and 2.8 gm. globulin, roentgenogram of the abdomen showed 3 gas distended small bowel loops. With a 19-gauge needle a paracentesis was performed which yielded 100 ml. of clear yellow fluid. Microscopic examination of this aspirate showed 800 cells per c.mm., 96% of which were lymphocytes. This patient improved slowly over one week's time on suction, antibiotics and corticosteroids, and could be discharged. This patient died in March, 1957, in uremia. Permission for necropsy was refused.

Symptoms referable to the central nervous system occurred in 17 patients. These are listed in Table 3. Convulsions occurred most frequently (7 patients); nonspecific psychiatric changes (depression, disorientation, delirium) were observed in patients not recently on corticosteroid therapy as the second most common type of neurologic finding. It is interesting to note that one patient was first seen with neurological signs clinically indistinguishable from Sydenham's chorea. The neurological manifestations of SLE have been discussed thoroughly by Clark and Bailey[3] in a recent article. It is not surprising that, in such a diffuse vascular disease as is SLE, an infinite variety of neurological signs might possibly be produced.

Comparison of the incidence of the changes in this series with other series reveals that, save for skin and cardiovascular findings, there is remarkably little difference.

LABORATORY FINDINGS. The most common laboratory change was moderate to severe anemia; hematocrits of less than 35% were found in 45 of our patients. The next most common hematological abnormality was elevation of the sedimentation rate. Every patient in our series presented an elevated sedimentation rate; 39 were 50 mm. per hour or more (Wintrobe method). A leukopenia of 4000 per c.mm. or less occurred in 27 patients on one or more occasions. The white cell count was quite variable in a given patient; bacterial infections proved capable of producing both leukocytosis and a shift to the left in the face of a pre-existing

leukopenia. Schilling differential counts on peripheral blood in leukopenic and nonleukopenic patients were unremarkable and, therefore, are not included in the tabulation (Table 4).

Thrombocytopenia was demonstrated in 4 patients all of whom clinically manifested purpuric skin lesions. It will be noted (see Table 3) that 2 patients who presented purpuric skin lesions did not have thrombocytopenia.

syndrome (Comens and Schroeder[4]), at any time. In 6 patients LE tests were not done (5 being prior to Hargraves's study) but pathologic studies at necropsy and the clinical picture were compatible with the diagnosis of SLE. In 4 patients, no true LE cells were found on numerous searches, the diagnosis being confirmed at necropsy in these cases. One patient is included in the face of numerous negative LE

TABLE 4.—LABORATORY CHANGES

| | *No. of Patients* | *% of Patients* | *Harvey 105 Pts.* % | *Dubois 62 Pts.* % | *Jessar 44 Pts.* % | *Total 258 Pts.* % |
|---|---|---|---|---|---|---|
| Urinary | | | | | | |
| Abnormal sediment | 44 | 94 | 65 | 56 | 64 | 68 |
| Albuminuria (1+) | 37 | 79 | — | — | 70 | 75* |
| Elevated NPN (50 mg./100 ml.) | 19 | 40 | 11 | — | 16 | 21† |
| Hematologic | | | | | | |
| Anemia (PCV 35%) | 45 | 96 | 78 | 78 | 95 | 85 |
| Elevated ESR (50 mm./hr.) | 39 | 83 | 93 | — | 86 | 89† |
| Leukopenia (WBC 4,000) | 27 | 57 | 68 | 68 | 70 | 66 |
| Thrombocytopenia | 4 | 8 | 26 | 10 | — | 17‡ |
| Positive LE Prep. | 36 | 88§ | 82 | 69 | — | 76‡ |
| Coombs Positive | 3 | 6 | — | — | — | — |
| Hyperglobulinemia (3.0 gm.%) | 38 | 81 | 58 | 27 | 82 | 58 |
| A/G Reversal | 35 | 75 | — | — | — | — |
| Bilirubinemia (1.5 mg./100 ml.) | 2 | 4 | 4 | 11 | — | 28‡ |
| Positive STS | 13 | 28 | 15 | 33 | 28 | 23 |

*Ninety-one patients.
†One hundred ninety-six patients.
‡Two hundred fourteen patients.
§This test was done in only 41 of the 47 patients.

Clotting times were normal in these patients; their purpura was presumably the result of increased vascular permeability.

Here as elsewhere, LE preparations became routine on suspected cases of SLE. Since Hargraves described the LE cell in 1948, using the peripheral blood (clotted) and bone marrow techniques (Gouldin and Diggs[8] and Haserick[13]), positive LE preparations were obtained in 36 patients, none of whom had received hydralazine, a compound known to produce a lupus-like

preparations because of a classical clinical picture of SLE and a positive renal biopsy.

CASE 3. M.A.S., a 41-year-old colored woman, was first admitted to John Gaston Hospital on October 13, 1953, with the chief complaint of a generalized body rash, of 2-weeks' duration. Four years prior to admission the patient had the gradual onset of a papular rash on her malar areas and nasal bridge. These slowly changed to erythematous macular areas which coalesced to form a classical "butterfly" eruption. Two weeks prior to admission, scaling erythematous lesions appeared over her body, arms and knees; the patient noticed slight fever and related

on questioning that her "joints had been stiff" for a brief time about 3 weeks prior to admission. Past history revealed that the patient had 3 convulsive seizures of unknown etiology 25 years prior to admission and was treated for syphilis 15 years prior to admission. Physical examination revealed temperature 99.4°, blood pressure 140/90, pulse 88, respiration 20. Moderate alopecia was present. Marked cervical, axillary and inguinal adenopathy was noted. A grade II apical systolic murmur was audible. The point of maximum intensity was felt in the fifth intercostal space at the mid clavicular line. The skin of the patient's neck was eroded and oozing and a scaling erythematous rash with marked depigmentation was present over the anterior and posterior chest, arms and knees in addition to a butterfly erythema of the face. Fundoscopic examination revealed narrowing of arterioles. Irregular areas of hyperpigmentation were noted on the tongue and buccal mucosa.

Laboratory examination revealed an erythrocyte count of 3.89 millon per c.mm., the hematocrit was 31%, the hemoglobin 8.5 gm.%, and the white blood count 5,100, the erythrocyte sedimentation rate was 57 mm. per hr. Urinalysis revealed the specific gravity to be 1.011, a trace of albumin, 1 RBC/HPF, 1 to 2 WBC/HPF, and 0 casts. The stained blood film showed hypochromic erythrocytes with anisocytosis, poikilocytosis and a normal leukocyte differential. Nonprotein nitrogen was 28 mg. per 100 ml., total proteins 6.6 gm.% (albumin 3.6 and globulin 3.0 gm.%). A serologic test for syphilis was negative Chest roentgenogram revealed normal heart and lung fields. Three LE preparations were negative. The patient's skin lesions were treated with lanolin, Benadryl and Atabrine. Her condition improved, and she was discharged to the clinic on the eighth hospital day.

Secondary infection of her skin lesions necessitated hospital care on 3 occasions following this admission. The status of her lupus was essentially unchanged but cortisone therapy had been instituted for the skin lesions. The patient had been maintained on cortisone, 15.0 mg. daily, but stopped medication one week prior to admission. Three hours prior to admission, the patient had a generalized convulsion and was given sodium amytal before her sixth admission on January 21, 1956. Blood pressure was 98/78, pulse 140, respirations 16 per min., and temperature 99°. The patient was stuporous. The skin lesions were improved and the erythematous depigmented lesions were quiescent. There was no adenopathy. The hematocrit was 44%, white blood cells numbered 29,950 per c.mm. with 91% polymorphonuclears. Urinalysis revealed a specific gravity of 1.022 with 4+ sugar, 1+ albumin, 5 to 7 leukocytes per high power field, 1 to 3 red blood cells per high power field, and an occasional granular cast (for the first time). Nonprotein nitrogen was 35 mg. per 100 ml., amylase 112 Somogyi units. The blood sugar was 780 mg. per 100 ml., $CO_2$ 7 mEq. per L. Urine culture grew out nonhemolytic *Staphylococcus aureus* and the cerebrospinal fluid examination was negative. Liver biopsy revealed nonspecific diffuse changes. The patient's diabetic acidosis was treated, and it was possible to maintain her on 65 units of NPH insulin daily. She was also treated with hydrocortisone, but this was discontinued prior to discharge on the thirtieth hospital day.

The seventh admission was again for pyoderma. A leukopenia of 3,800 per c.mm. was recorded. Culture of skin lesions revealed hemolytic *Staphylococcus aureus*, coagulase positive. She was treated with cortisone and antibiotics; the skin lesions responded well. Her insulin requirement gradually diminished, and the patient was discharged with sugar-free urine on no insulin at all. The eighth and last admission was on July 26, 1956, again for pyoderma due to *Staphylococcus aureus*. The LE cell preparation was again negative. Punch renal biopsy was performed revealing hematoxylin bodies in the section thus confirming for the first time the diagnosis of SLE rather than disseminated discoid LE. The patient was again complaining of multiple arthralgia without objective change; she improved on antibiotics and was discharged on the thirty-second hospital day without medication.

Bone marrow aspirations were performed on 30 patients; 19 of these revealed LE cells as the only abnormal finding. In 7 marrows there was an increased number of plasma cells and histiocytes as noted by Michael *et al.*[21]. A positive reaction to serologic test for syphilis was encountered 13 times in the absence of history of lues. In 38 instances serum globulins were elevated above 3.0 gm.%; the albumin-globulin ratio was reversed in 35 cases. Abnormal urinary findings were prevalent. A highly persistent observation was the

presence of a moderate number of erythrocytes, leukocytes, granular and hyaline casts in the urinary sediments. Of somewhat less frequent occurrence was proteinuria greater than one plus. Uremia, manifesting itself by an elevated nonprotein nitrogen, supervened at some time in the course of 19 patients. It is apparent from the findings in this report as well as from the findings in other reports, that with the exception of the LE cell test the laboratory abnormalities are nonspecific and therefore furnish only corollary diagnostic evidence. The incidence of specific laboratory abnormalities varies slightly from series to series. Lymph node, muscle, and skin biopsies were of limited diagnostic assistance in this series. Of 11 skin biopsies, only 6 showed changes compatible with lupus. All 4 muscle biopsies were negative. Of 16 lymph node biopsies only 3 microscopically suggested the diagnosis of SLE by showing focal areas of necrosis, perivascular infiltrates of inflammatory cells or fibrinoid degeneration of the vessel walls. Renal biopsies were performed on only 2 patients, both still living. One is described above (Case 3). The other renal specimen showed only changes of chronic pyelonephritis and early arteriosclerosis.

TABLE 5.—IMMEDIATE CAUSES OF DEATH

| *Cause* | *No. of Cases* |
|---|---|
| Bronchopneumonia | 11 |
| Cardiac failure | 3 |
| Uremia | 3 |
| Central Nervous System | 3 |
| Shock (Due to GI hemorrhage) | 1 |
| Unknown | 2 |

PATHOLOGY. To our certain knowledge, 23 deaths have occurred among our patients. In 18 cases permission for necropsy was obtained. Immediate causes of death are listed in Table 5.

In evaluating all pathological findings, the criteria of Klemperer and coworkers were used[1,16,17,18]. A summary of significant changes is presented in Table 6. Those changes listed under "classical" are those changes described by Klemperer and others as typical of SLE, for example, "wire-looping" of glomeruli, perivascular "onion-skin" lesion in spleens, hematoxylin bodies, and others. Nonspecific findings are those abnormalities not particularly indicative of lupus erythematosus. No unusual pathologic changes were demonstrated in these cases; the distribution of the lesions observed documents once more the widespread nature of this disease manifested by diffuse collagen degeneration and disseminated vasculitis.

COURSE AND PROGNOSIS. It is general knowledge that the course of SLE is highly variable and marked by spontaneous remissions and exacerbations. The patients in this series in no wise deviate from that pattern. Our most fulminating case had a course of only 6-weeks' duration apparently not affected by what were thought to be adequate doses of corticosteroids. On the other hand, one case survived more than 25 years on first, gold, then later Atabrine. Corticosteroid therapy has been employed for SLE since Grace and Combes[9] described its use in 1949. It is believed that by the use of corticosteroids, remissions have been induced. Our experience, with few exceptions, confirms this. In our hands Atabrine has been effective in producing remissions in mild cases and has seemed to prolong remissions. Dubois[6] recently described in detail the therapy of SLE; our treatment conforms in general to the principles described in his article.

**Summary.** Systemic lupus erythematosus is now being recognized as frequently as pernicious anemia and acute glomerulonephritis.

Of our patients 85% were Negroid and 93% females. The disease usually started in the third decade with arthralgia and skin eruption as the most common initial manifestations.

At some time during the course of the disease, fever occurred in all cases and some form of reticuloendothelial system involvement presented in 98%. Weight loss, arthralgia or arthritis, skin and mucous membrane involvement were found in 85 to 90% of cases. Only slightly less often were serous membranes and the cardiovascular system affected. Manifestations referable to the gastrointestinal system were found in over one-half of patients while the central nervous system was affected in over one-third.

Anemia was demonstrated in 96% of cases while urinary sediment was abnormal in 94%. The LE cell test was done on 41 patients and 88% of these were positive. Albuminuria, elevated sedimentation rate, and hyperglobulinemia were found in about 80% of patients.

Cases are presented which illustrate:

TABLE 6.—PATHOLOGICAL CHANGES IN 18 NECROPSIES

| | | *Number of Cases* | *% of Cases* |
|---|---|---|---|
| Skin | Classical | 7 | 39 |
| | Nonspecific | 3 | — |
| Lymph Nodes | Classical | 7 | 39 |
| | Nonspecific | 7 | — |
| Liver | Classical* | 4 | 22 |
| | Nonspecific | 14 | — |
| Spleen | Classical | 12 | 67 |
| | Nonspecific | 3 | — |
| Kidney | Classical | 13 | 72 |
| | Nonspecific | 5 | — |
| Heart | Classical† | 6 | 33 |
| | Nonspecific | 3 | — |
| | Libman-Sacks | 5 | 28 |
| Serous membranes | Pleurisy | 17 | 94 |
| | Pericarditis | 15 | 83 |
| | Peritonitis‡ | 10 | 55 |
| Lungs | Classical§ | 9 | 50 |
| | Nonspecific | 9 | — |
| Central nervous system | Vascular changes | 7 | 39 |
| Generalized vasculitis | | 12 | 67 |

*Focal necrosis, vascular lesions, fibrinoid degeneration or hematoxylin bodies.

†Mural endocarditis, hematoxylin bodies, fibrinoid degeneration (Libman and Sacks[19], Montgomery[22]).

‡Serous or fibrinous peritonitis as manifested by ascites or adhesions.

§Harvey: Involvement of alveolar hyaline membrane.
Basophilic mucinous edema of alveolar walls (Talbot and Ferrandis[23]).
Capillary thrombi (Harvey *et al.*[11]).

1) onset of SLE with hemorrhage due to thrombocytopenic purpura, 2) known SLE simulating an acute abdomen, and 3) diagnosis by renal biopsy in the presence of numerous negative LE cell preparations.

Clinical courses were variable. Bronchopneumonia most frequently caused death. Necropsy findings were not unusual.

In comparison with other large series it was found that skin hyperpigmentation was more frequent in this group and photosensitivity occurred less often. Cardiovascular complications were much more common in this series. These differences probably reflect in part, 1) the tendency of dark skins to respond to any trauma with hyperpigmentation, and 2) the known prevalence of hypertensive cardiovascular disease in American Negroes.

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## SUMMARIO IN INTERLINGUA

### Systemic Lupus Erythematose: Un Reporto Clinic De 47 Casos, Con Presentation Del Constatationes Pathologic Facite Al Necropsia In 18 De Illos


Al tempore presente, le recognition de systemic lupus erythematose es tanto frequente como le recognition de anemia perniciose e de glomerulonephritis acute.

Inter nostre patientes, 85% esseva negroide, e 93% esseva femininas. Le morbo


XUM

comenciava usualmente durante le tertie decennio del vita del patiente. Arthralgia e eruptiones cutanee esseva le plus commun manifestationes initial.

A un certe tempore in le curso del morbo, febre occurreva in omne le casos. Le systema reticulo-endothelial esseva afficite in un maniera o un altere in 98% del casos. Perdita de peso, arthralgia o arthritis, e affection de pelle e membrana mucose esseva trovate in 85 a 90% del casos. Solmente pauco minus frequente esseva affectiones de membrana mucose e de systema cardiovascular. Manifestationes referibile al systema gastrointestinal esseva trovate in plus que un medietate del patientes, durante que le systema nervose central esseva afficite in plus que un tertio.

Anemia esseva demonstrate in 96% del casos, durante que le sedimento urinari esseva anormal in 94%. Le test pro cellulas "L.E." esseva executate in 41 patientes. In 88% le test esseva positive. Albuminuria, sedimentation accelerate, e hyperglobulinemia esseva trovate in circa 80% del patientes.

Es presentate casos individual pro illustrar (1) le declaration de systemic lupus erythematose con hemorrhagia in consequentia de purpura thrombocytopenic, (2) le simulation de abdomine acute per systemic lupus erythematose de identitate establite, e (3) le diagnose per biopsia renal in le presentia de numerose negative preparatos de cellulas "L.E."

Le cursos clinic esseva variabile. Bronchopneumonia esseva le plus frequente causa de morte. Le constatationes necroptic non esseva inusual.

In comparation con altere grande series, il esseva constatate in le presente gruppo que hyperpigmentation cutanee esseva plus frequente e que photosensibilitate occurreva minus frequentemente. Complicationes cardiovascular esseva multo plus commun in iste serie. Iste differentias es probabilemente reflexiones, al minus in parte, de (1) le tendentia in pelles obscur de reager a omne trauma per hyperpigmentation e (2) le cognoscite prevalentia de hypertensive morbo cardiovascular in negros american.

## SUMMARIO IN INTERLINGUA

*(See page 311 for original article)*

### Le Constituentes Chimic De Polline De Pino E Lor Relation Possibile Con Sarcoidosis

1. Ha essite demonstrate que polline de pino possede characteristicas de tincturation acido-resistente, simile al bacillo tubercular.

2. Esseva trovate, como constituentes de polline de pino, un lipido acido-resistente e un amino-acido con le characteristicas de acido alpha-epsilon-diamino-pimelic.

3. Polline de pino es capace a evocar granulomas de cellulas epithelioide in animales normal. Lesiones plus diffuse es trovate in animales que es hypersensibile a tuberculina e in animales normal que ha recipite injectiones de fractiones chimic derivate ab polline de pino.

# A CLINICAL EVALUATION OF THE PARENTERAL USE OF NOVOBIOCIN

By Mark W. Garry, M.D.

ASSOCIATE PROFESSOR OF MEDICINE, MARQUETTE UNIVERSITY SCHOOL OF MEDICINE AND CHIEF, MEDICAL SERVICE, VETERANS ADMINISTRATION HOSPITAL, WOOD (MILWAUKEE), WISCONSIN

Novobiocin* is a relatively new antibiotic, having been introduced at the Third Annual Antibiotic Symposium in Washington, D.C. in November, 1955, as streptonivicin. It is derived from the actinomycete, *Streptomyces niveus.*

Numerous publications (David and Burgner[1], Jawitz, Bertie and Sonne[2], Kirby, Hudson and Noyes[3], Limson and Romansky[4], Lin and Coriell[5], Martin *et al.*[6], Meyer and Ward[7], Morton, Prigot and Maynard[8], Mullins and Wilson[9], Nichols and Finland[10], Petersdorf, Curtin and Bennett[11], Pulaski and Isokane[12], Rantz *et al.*[13], Rutenburg, Shapiro and Schweinburg[14], and Schneierson and Amsterdam[15]) have attested to its activity *in vitro* and *in vivo* against many Gram-positive and a few Gram-negative bacteria. These evaluations of the orally administered monosodium salt of novobiocin have indicated its clinical efficiency.

Parenteral administration of novobiocin has been reported only in occasional cases (David and Burgner[1] and Kirby, Hudson and Noyes[3]). It is the purpose of this paper to describe the intramuscular or intravenous administration in 17 patients.

**Material and Methods.** CHOICE OF PATIENTS. The 17 patients studied were all critically ill and the infections for the most part were complications either of surgery or pre-existing disease. The site of infection was the urinary tract in 8 patients, the lungs in 3, the gastrointestinal tract in 3, and 3 times in wounds.

DOSAGE. Novobiocin was administered intravenously or intramuscularly in doses of 1 or 2 gm. daily. The intervals between doses varied from 4 to 12 hours. The most frequently used dosage schedule was 500 mg. every 6 hours. Parenteral administration was chosen either because the patient was unable to tolerate oral medication or an immediate high therapeutic level was desired.

PREPARATION OF MATERIAL. 1) For Intravenous Use: A solution was prepared by adding 5 ml. of sterile solution N, N-Dimethylacetamide, 10%, to the vial containing sterile novobiocin, 500 mg. This concentrated solution was added to 500 or 1000 ml. of isotonic sodium chloride solution and administered by continuous drip infusion. 2) For Intramuscular Use: A concentrated novobiocin solution was prepared by the addition of 3 ml. of sterile N, N-Dimethylacetamide, 10%, to the vial containing 500 mg. of the sterile antibiotic. Solution was obtained promptly by gentle agitation and the material was injected deeply into the gluteal muscle; when administered slowly there was no complaint of pain or distressing local reaction.

BACTERIOLOGY (TABLE 1). Sixteen blood cultures were performed on 9 patients, 5 of which were positive; the bacterial flora included *Paracolobactrum coliforme,* 1, *Staphylococcus aureus* (virulent), 2, Proteus, 1, and *Klebsiella (aerobacter aerogenes),* 1. Four of these organisms were sensitive to novobiocin: *Paracolobactrum coliforme,* 1, *Staphylococcus aureus* (virulent), 1, Klebsiella, 1, and Proteus, 1.

Twenty-three urine specimens obtained on 17 patients were cultured; 12 were positive: *Staphylococcus aureus* (vir.), 2, *E. coli,* 1, Klebsiella, 5, Proteus, 2, and *Pseudomonas aeruginosa,* 2. Seven of these were sensitive to novobiocin.

*The novobiocin used in this study was generously furnished by the Upjohn Company (trade name Albamycin).

TABLE 1.—CULTURE CHARACTERISTICS

| | No. of Cultures | Positive Cultures | Organism | No. Sensitive to Novobiocin | Organism |
|---|---|---|---|---|---|
| Blood | 16 | 5 | *Paracolobactrum coliforme* —1<br>*Staphylococcus aureus* (vir.)—2<br>Proteus —1<br>*Klebsiella* (*aerobacter aerogenes*) —1 | 4 | *Paracolobactrum coliforme* —1<br>*Staphylococcus aureus* (vir.)—1<br>Proteus —1<br>Klebsiella —1 |
| Urine | 23 | 12 | *Staphylococcus aureus* (vir.)—2<br>*E. coli* —1<br>Klebsiella —5<br>Proteus —2<br>*Pseudomonas aeruginosa* —2 | 7 | Klebsiella —3<br>Proteus —2<br>*E. coli* —1<br>*Paracolobactrum coliforme* —1 |
| Sputum | 7 | 7 | *Staphylococcus aureus* (avir.)—1<br>*Staphylococcus aureus* (vir.) —3<br>Klebsiella —1<br>Proteus —1<br>*Candida albicans* —1 | 7 | |
| Wound | 4 | 3 | *Staphylococcus aureus* (avir.)—1<br>*Staphylococcus aureus* (vir.) —1<br>*E. coli* —1 | 3 | |
| Nasopharynx | 1 | 1 | *Paracolobactrum coliforme* —1 | 1 | |
| Pleural Fluid | 1 | 1 | *E. coli* —1 | 1 | |
| Stool | 3 | 1 | *Staphylococcus aureus* (avir.)—1 | 1 | |

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| Sputum | 7 | 7 | *Staphylococcus aureus* (avir.) —1<br>*Staphylococcus aureus* (vir.) —3<br>Klebsiella —1<br>Proteus —1<br>*Candida albicans* —1 | 7 | |
| Wound | 4 | 3 | *Staphylococcus aureus* (avir.) —1<br>*Staphylococcus aureus* (vir.) —1<br>*E. coli* —1 | 3 | |
| Nasopharynx | 1 | 1 | *Paracolobactrum coliforme* —1 | 1 | |
| Pleural Fluid | 1 | 1 | *E. coli* —1 | 1 | |
| Stool | 3 | 1 | *Staphylococcus aureus* (avir.) —1 | 1 | |

TABLE 2.—SUMMARY OF PATIENTS TREATED WITH NOVOBIOCIN

| | Case | Age | Diagnosis | Organism | Source | Parenteral Dosage in Hours | Days of Rx | Result | Toxicity |
|---|---|---|---|---|---|---|---|---|---|
| W.W. | 1 | 64 | Renal failure following total cystectomy and penectomy for carcinoma | *Paracolobactrum coliforme* | Blood | 500 q 12 | 4 | Died | None |
| W.W. | 2 | 61 | Pyelonephritis | Klebsiella | Urine | 500 q 12 | 5 | Excellent | None |
| G.P. | 3 | 68 | Massive bronchopneumonia with Emphysema | *Staphylococcus aureus* (vir.) | Sputum | 500 q 6 | 3 | Died | None |
| R.S. | 4 | 20 | Abscess and peritonitis following colectomy for ulcerative colitis | *Staphylococcus aureus* (vir.)<br>*E. Coli* | Urine | 500 q 12 | 19 | Died | None |
| B.J. | 5 | 45 | Carcinoma of esophagus | Pneumococcus<br>*Staphylococcus aureus* (vir.) | Blood | 500 q 6<br>250 q 6 | 58<br>2 | Good | None |
| M.H. | 6 | 61 | Chronic cystitis | Klebsiella | Urine | 500 q 6 | 6 | Died | None |
| C.H. | 7 | 60 | Carcinoma of anus with metastasis pyelonephritis | Proteus<br>*Staphylococcus aureus* (vir.) | Blood, urine, nasopharynx colostomy | 500 q 6 | 3 | Died | None |
| A.G. | 8 | 41 | Mycotic aneurysm, left common carotid artery, acute cholecystitis | Proteus | Blood | 1000 q 12 | 10 | Excellent | None |
| F.F. | 9 | 58 | Wound infection | Beta hemolytic streptococcus | Wound | 250 q 6 | 19 | Excellent | None |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| F.F. | 9 | 58 | Wound infection | Beta hemolytic streptococcus | Wound | 250 q 6 | 19 | Excellent | None |
| H.E. | 10 | 68 | Squamous cell carcinoma of mouth, radical neck dissection | *Staphylococcus aureus* (vir.) | Wound | 1000 q 12 | 2 | Excellent | None |
| C.B. | 11 | 37 | Carcinoma of lung, pyelonephritis, amyloidosis | Klebsiella | Urine | 250 q 4 | 1 | Died | None |
| R.B. | 12 | 69 | Pyelonephritis, acute renal failure | *Pseudomonas aeruginosa*, *Staphylococcus aureus* (vir.) | Blood | 500 q 6<br>250 q 6 | 3<br>1 | Died | None |
| D.B. | 13 | 58 | Pneumonia, bronchogenic carcinoma | *Staphylococcus aureus* (vir.) | Sputum | 250 q 6 | 18 | Excellent | None |
| L.H. | 14 | 46 | Third degree burns lower extremities and abdomen, infected graft | Proteus | Wound | 250 q 6 | 10 | Excellent | None |
| K.H. | 15 | 35 | Fulminating ulcerative colitis, acute pyelonephritis colectomy | *Staphylococcus aureus* (vir.) Klebsiella, *Paracolobactrum coliforme* | Stool | 500 q 6 | 3 | No Response | None |
| G.H. | 16 | 79 | Carcinoma of rectum, cystitis Anterior resection | Proteus *Staphylococcui aureus* (vir.) | Urine Rectal | 500 q 12 | 5 | Excellent | None |
| J.K. | 17 | 61 | Benign prostatic hypertrophy prostatectomy, bacteremia | Klebsiella | Urine Blood | 500 q 12 | $2\frac{1}{2}$ | Died | None |

Cultures of 7 sputum specimens from, 6 patients were positive: *Staphylococcus aureus* (avir.), 1, *Staphylococcus aureus* (vir.), 3, Klebsiella, 1, Proteus, 1, and *Candida albicans*, 1. All 7 were sensitive to novobiocin.

Wound cultures were obtained in 4 cases, 3 were positive: *Staphylococcus aureus* (avir.), 1, *Staphylococcus aureus* (vir.), 1, and *E. coli*, 1. All 3 were sensitive to novobiocin.

Five cultures taken from the nasopharynx, pleural fluid and stool showed growth on 3 plates, one from each source. *Paracolobactrum coliforme* recovered from the nasopharynx, *E. coli* from the pleural fluid and *Staphylococcus aureus* (avir.) from the stool were all sensitive to novobiocin.

**Results.** The clinical responses to parenteral novobiocin therapy are illustrated in Table 2. It must be remembered that these were desperately ill patients to whom this form of medication was offered as the only hope for survival. Of the 17 patients, 7 showed an excellent response with prompt fall in body temperature and subsidence of all evidences of infection; one other survived, but his course assumed a subacute character for several weeks until ultimate elimination of the infecting organisms was accomplished.

Eight of the 9 patients, who did not respond to therapy with novobiocin, died with overwhelming infection; one, who did not respond to treatment, survived following total colectomy which was performed 8 days after the start of novobiocin. The course of treatment varied from 1 to 60 days with an average of 10 days.

There was no evidence of adverse reaction due to the drug in any of the patients treated nor was there any excessive pain on intramuscular injection. Although skin eruptions have been reported with the oral preparation, they were not encountered in this group of patients.

**Discussion.** Parenteral administration has been used in a total of 17 patients; in 8 cases the intravenous route was chosen, another 8 patients received the medication intramuscularly and in one case both routes were utilized.

Nine patients with severe staphylococcal infection were treated; 4 survived; 3 of whom showed a prompt response while the fourth, who had a complicating pneumococcal infection, required a prolonged course of therapy before successful termination. Of the 5 patients who died, death occurred in 3 cases within 72 hours of the start of treatment; one case with staphylococcal septicemia failed to survive in spite of sterilization of the blood stream, and one case with fulminating ulcerative colitis did not respond after 8 days of intensive medication.

Four patients with Klebsiella infection of the genitourinary tract were treated. Three of the 4 patients died, but only 2 of the cases could be considered to have had adequate therapy; one, treated for 5 days showed an excellent response, the other died on the sixth day.

Two patients in whom blood culture revealed *Proteus vulgaris* were given novobiocin intravenously. One case, with acute cholecystitis and mycotic aneurysm of the left common carotid artery, improved substantially within 48 hours with lysis of the fever and clearing of the sensoria and was free of infection by the tenth day. The other case, with Proteus bacteremia, developed a complicating bowel obstruction on the third day of therapy and died following operation.

One patient with *Paracolobactrum coliforme* infection and one with hemolytic streptococcal infection had satisfactory outcomes.

With the variety of clinical patterns, the diversity of complicating factors, and the uneven range of therapeutic exhibition, it is difficult to present a clear evaluation of the therapeutic effectiveness of parenteral novobiocin.

Six of the 9 failures were treated for less than 72 hours, an insufficient period of therapy. Aside from these inadequately treated cases, there were 8 satisfactory responses and 3 therapeutic failures. It would appear, therefore, that from this limited experience with 17 desperately ill patients, parenteral novobiocin is therapeutically effective and offers a reasonable expectation of a favorable response even in seemingly hopeless cases.

**Summary and Conclusions.** Novobiocin was administered parenterally to 17 seriously ill patients. Nine patients died and 8 patients survived. Of the 9 patients who died, 6 are felt to have had an inadequate course of therapy. Seven of the 8 who survived had an excellent clinical response marked by prompt defervescence and improvement in their general condition. The drug in parenteral form was well tolerated. The parenteral preparation of novobiocin extends still further its proven value in the control of infection.

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## SUMMARIO IN INTERLINGUA

### Un Evalutation Clinic Del Uso Parenteral De Novobiocina


Novobiocina esseva administrate parenteralmente a 17 patientes seriemente malade. Octo superviveva e 9 moriva. Ex le 9 qui moriva, 6 recipeva un curso therapeutic que debe esser considerate como adequate. Septe del 8 superviventes monstrava un excellente responsa clinic, marcate per prompte defervescentia e melioration del condition general. Le droga esseva ben tolerate in administration parenteral. Le preparato al uso parenteral extende additionalmente le provate valor de novobiocina in le subjugation de infectiones.

# THE COMPARATIVE EFFECT OF BRONCHODILATOR AGENTS ADMINISTERED BY INHALATION: A CONTROLLED CLINICAL STUDY

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The administration of a bronchodilator agent by inhalation has been suggested as an effective method for relieving bronchospasm (Lukas[3]). A number of nebulizing devices are available, and this method of treatment is being used more frequently in the management of obstructive pulmonary disease. As the number of bronchodilator drugs available for inhalation therapy is increasing, it becomes important to have reliable information concerning the activity of these drugs. Accordingly, this study was undertaken to determine the comparative effectiveness of several bronchodilator agents administered by inhalation to patients with asthma and pulmonary emphysema. A further objective was to present a method for the controlled evaluation of several bronchodilator drugs in man.

A number of objective measurements have been employed to delineate the effects of bronchodilator drugs. The vital capacity, the timed expiratory capacity, and the maximum breathing capacity have been the most widely used of these tests (Cander and Comroe[2]). The *vital capacity* is the amount of air which can be moved with one maximal expiratory effort. The *timed expiratory capacity* takes velocity into account, and usually is expressed as the per cent of the vital capacity moved in the first second or the first three seconds of a single maximal expiration. The *maximal breathing capacity* is the amount of air that can be moved in one minute. This is an over-all measurement of ventilation. These tests have been standardized and the normal values can be predicted from the patient's age, sex, and body size (Baldwin, Cournand and Richards[1], Gaensler[4]).

Each of these tests of ventilation is affected by bronchospasm; however, each is not always affected to the same degree (Cander and Comroe[2]). Therefore, it seemed advisable to use a combination of these procedures to determine an *index of functional impairment*. Acute changes in the index following administration of the test materials then are used to represent bronchodilator activity.

A major difficulty in evaluating drug activity is the variability between patients. This difficulty may be overcome by averaging the individual variations of a large number of persons. This procedure frequently is time-consuming and is not always satisfactory; this is particularly so when the relative activity of several drugs is being compared. The Latin square design utilized in this

investigation is helpful in isolating these variations. This arrangement permits statistically significant data to be secured from a minimal number of patients as each person serves as his own control and also as a control for the remainder of the group.

The bronchodilator activity of the following agents was compared. Normal saline was included as a control.

**Material and Methods.** Sixteen patients, hospitalized because of chronic obstructive pulmonary disease, were selected for study. Seven patients had asthma, 3 had pulmonary emphysema, and 6 had combined asthma and pulmonary emphysema. Eight of the patients had concomitant heart disease; however, none was in failure. All of the patients had typical physical and Roentgen evidence of their pulmonary disease. These findings were substantiated by ventilatory studies. All of the patients were men and their ages ranged from 47 to 76 years.

TABLE 1.—EXPERIMENTAL GROUPS

(Summary of Data)

| *Group* | *Patient* | *Age* | *Diagnosis* | *Patient's Drug of Choice* | *Drug Showing Most Bronchodilator Action* | *Adverse Reactions* |
|---|---|---|---|---|---|---|
| I | 1 | 68 | Asthma, emphysema, cor pulmonale | None | A | None |
| | 2 | 69 | Emphysema, bronchitis, ASHD | None | C | None |
| | 3 | 60 | Emphysema, ASHD | C | A | None |
| | 4 | 61 | Emphysema, HCVD, asthma | B | B | None |
| II | 5 | 63 | Emphysema, bronchitis, ASHD | A | A | None |
| | 6 | 64 | Asthma, cirrhosis | A or D | C | None |
| | 7 | 61 | Asthma, emphysema, bronchitis | C | B | "Dizzy," Drug A |
| | 8 | 47 | Asthma | B or C | A | None |
| III | 9 | 63 | Asthma, ASHD | C | A | None |
| | 10 | 71 | Asthma, HCVD | None | A | "Dizzy," Drug A |
| | 11 | 76 | Asthma, emphysema, dermatitis | A | C | None |
| | 12 | 67 | Emphysema, asthma, HCVD | A | A | None |
| IV | 13 | 49 | Asthma | A | C | None |
| | 14 | 66 | Asthma, emphysema | D | C | None |
| | 15 | 59 | Asthma | C | A | None |
| | 16 | 51 | Asthma | D | C | None |

Drug A: Isoproterenol HCl 1:200 (Isuprel).

Drug B: A mixture of Cyclopentamine HCl, Aludrine HCl, and Procaine HCl 1:200 (Aerolone).

Drug C: An N substituted Arterenol derivative 1:200 JB 251).*

Drug D: Normal saline.

The patients were divided at random into 4 groups (Table 1). Each was studied on 4 consecutive days, and a different test was given each day. The drugs were administered according to a Latin square schedule (Table 2). In this way, each patient in the group received the drugs in a different order; each drug was given every day; and each patient received every drug.

Pulmonary function studies were performed before the administration of each test sub-

*Supplied by Lakeside Laboratories, Milwaukee, Wisconsin.

stance and 10 minutes after 0.5 ml. of the agent was given by hand nebulizer. Other medications were withheld for at least 6 hours prior to each test procedure.

The *vital capacity*, *maximum breathing capacity*, and the *3-second expiratory capacity* before and after the administration of each drug were calculated as the per cent of the predicted value, using the formula of Baldwin, Cournand and Richards[1], and of Gaensler[4]. The average of these values before administration of the test substance was used as an *index of the functional impairment* (FI INDEX).

Bronchodilator activity was recorded as the change in the FI INDEX following administration of the drugs. This change was designated the *bronchodilator index* (BD INDEX). To avoid negative numbers in the statistical analysis, 20 was added to each BD INDEX value.

The FI INDEX was chosen to express *functional impairment* for several reasons. The vital capacity, maximum breathing capacity, and timed expiratory capacity, all are affected by bronchial or bronchiolar obstruction (Cander and Comroe[2]). By relating each of these determinations to the predicted value, the results were corrected for age, sex, and body size. The FI INDEX reflects changes in pulmonary function other than bronchospasm. These include loss of elastic tissue, and permanent lung damage. These changes, however, are not likely to be modified by administration of the test substances. Therefore, acute change in this index should reflect bronchodilatory activity. An additional advantage is secured by recording bronchodilator activity in this manner. This procedure permits the BD INDEX to be independent of the initial ventilatory values, and it measures only the change following therapy.

**Results.** The BD Indexes for the test materials used in the 4 groups of patients were combined and subjected to an analysis of variance.* The results are summarized in Table 3. The variation in the BD Index between the patient and the variation due to the different drugs showed a significant F value. This indicates a statistically significant difference in the response of the individual patient to therapy and in the average response of all patients to the various test materials.

As expected, the data showed a statistically significant variation in the response of the individual patient to the test substances. This confirmed the often repeated observations that patients differ one from another. While each person had his own pattern of

TABLE 2.—SCHEDULE OF DRUG ADMINISTRATION FOR EACH GROUP OF PATIENTS

| | *Patient* | *1* | *2* | *3* | *4* |
|---|---|---|---|---|---|
| DAY OF STUDY | I | A | C | D | B |
| | II | B | A | C | D |
| | III | C | D | B | A |
| | IV | D | B | A | C |

Letters in body of table indicate test substance given. See text for identification.

*Both the FI INDEX and the BD INDEX values were distributed normally.

response to therapy, this pattern did not show a significant variation during the period of study. There was a day-to-day variation in each patient's FI Index. This is in conformity with studies reported by Gaensler[4]. This variation, however, was a random one, and the experimental design was arranged to minimize its effect on the final results.

BD Index for the control material D. While the data do not show a statistically significant difference between the mean BD Indexes for each of these drugs, it is interesting that 8, or 50%, of the patients received the most objective improvement with drug A.

The patients were asked to pick the drug which gave them the most subjective relief. The results are summarized in Table 1. Three of the 16 patients were unable to differentiate between the effects of any of the test substances, and 2 could not decide between two drugs.

It is interesting that only 3, or approximately 19%, of the patients were able to pick the drug which gave the best objective response. Three patients erroneously picked the control substance, normal saline, as giving them the most subjective relief. These find-

TABLE 3.—ANALYSIS OF VARIANCE BRONCHODILATOR INDEX

| *Source of Variance* | *D.F.* | *S.S.* | *M.S.* | *F* |
|---|---|---|---|---|
| Between Patients | 15 | 1,141.9486 | 76.1299 | 2.5613* |
| Between Days | 3 | 19.0104 | 6.3368 | 0.2131 |
| Between Treatments | 3 | 359.1004 | 119.7001 | 4.0272† |
| Residue | 42 | 1,248.3606 | 29.7228 | — |
| TOTAL | 63 | 2,768.4200 | — | — |

*Significant at 5% level.
†Significant at 1% level.

TABLE 4.—BRONCHODILATOR INDEX FOR EACH TEST MATERIAL AND COMPARISON OF DIFFERENCE BETWEEN INDEXES BY STUDENT-NEWMAN-KEULS MULTIPLE RANGE TEST

| *Mean Bronchodilator Index for Each Drug* | *Difference Between Means* | $T_R$* | *Tabular Value P=0.05* |
|---|---|---|---|
| A=6.6 | A—D=6.3 | 4.62 | 3.79 |
| B=4.7 | B—D=4.4 | 3.32 | 2.86 |
| C=5.2 | A—B=1.9 | 1.39 | 3.44 |
| D=0.3 | — | — | — |

$$*T_R = \frac{X\ \max - X\ \min}{\sqrt{\frac{S^2}{N}}}$$

$S^2 = 29.7228$

$N = 16.0$

The average response of the patients to the test materials was then examined by means of the Student-Newman-Keuls Multiple Range Test (Table 4). This procedure is useful in testing significant differences between means which have the same standard error and are obtained from the same number of observations (Federer[3]).

It is evident that the mean BD Indexes for drugs A, B, and C are significantly different from the mean

ings emphasize the hazards of relying on subjective reports and observations in the evaluation of therapeutic action.

Two patients complained of some dizziness following the administration of drug A. No other adverse effects were noted from any of the test substances.

**Summary and Conclusions.** This clinical study was undertaken to: (1) determine the effectiveness of 3 bronchodilator drugs administered by inhalation to patients with chronic asthma or pulmonary emphysema, or both, (2) present an improved method for the comparative evaluation of several bronchodilator drugs. A Latin square experimental design was utilized to secure statistically significant data from a minimal number of patients.

*Bronchodilator activity*, the BD Index, was measured as the change in the FI Index following administration of the test drugs. The *degree of functional impairment*, the FI Index, was recorded as the average per cent of predicted values for the vital capacity, maximum breathing capacity, and 3-second expiratory capacity.

The following conclusions appear justified:

1. The 3 drugs utilized in the study have a better bronchodilator action than the control substance, normal saline.

2. Eight, or 50%, of the patients had their best objective response to drug A (Isuprel). The data, however, did not show a statistically significant difference between the average bronchodilator action of the 3 drugs utilized in the study (Isuprel, Aerolone, and J B 251).

3. Mild "dizziness," the only side effect, was observed in 2 patients following administration of drug A.

4. Thirteen, or 81%, of the patients were unable to pick the drug which gave them the best objective results.

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*(Interlingua on page 368)*

# MARKED AND SUSTAINED BLOOD CHOLESTEROL LOWERING EFFECT BY MEDICATION WITH NIACIN AND PYRIDOXINE

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Altschul, Hoffer and Stephen[1] reported in 1955 the original finding of hypocholesterolemic effect of large doses of nicotinic acid in man. Their observation found immediate clinical application by Parsons, Jr. and his associates at the Mayo Clinic[5], who administered daily doses of 3 to 6 gm. nicotinic acid to 20 patients with hypercholesterolemias of various types and obtained marked and sustained decreases in blood cholesterol levels in all instances.

The great clinical implications of these findings are obvious. Any agent which promises to induce a lowering of the blood cholesterol level deserves thorough investigation, in view of the role which hypercholesterolemia is likely to play in the development of atherosclerosis. Attempts to elucidate the basic mechanism of this hitherto unknown action of nicotinic acid are needed as much as further extension of the clinical application.

The present study was undertaken to add such observations. While it was in progress, a second report was published by Parsons and Flinn[6], extending and confirming their previous observations. Our own findings are in full agreement with these reports and give further evidence of the efficacy of nicotinic acid medication in lowering elevated blood cholesterol levels. They also add information about the effect of these large doses of niacin on liver function and carbohydrate metabolism, and about an apparent potentiation of the effect of niacin by combining it with pyridoxine.

**Materials and Methods.** THE PATIENT MATERIAL. Fifty-three patients were studied, 45 were inpatients of the Jewish Chronic Disease Hospital and 8 were outpatients. Thirty-five of the patients were females and 18 males. Their ages varied between 30 and 96 years with an average age for the female group of 63 years and for the males of 56 years. All patients had clinical evidence of advanced degenerative vascular diseases; most had hypertension; 8 had in addition arteriosclerotic Parkinsonism; 10 had diabetes mellitus; 12 were hemiplegics; 3 were hypothyroids and one suffered from familial hypercholesterolemia and hyperlipemia. All patients were well known to us for at least one year and their serum cholesterol level had been established and checked by repeated examinations. In all but 5 patients the serum cholesterol level was above 250 mg. per 100 ml. Twenty of these patients have been under continuous treatment for more than 10 weeks. Others have been either under interrupted treatment, or have been on medication for less than 10 weeks.

THE MEDICATION. In a preliminary group nicotinic acid in capsules of 50, 100, and 500 mg. each was used, 1 to 3 t.i.d. Many patients objected to this treatment, not only because of the attendant flushing, but also because it involved the ingestion of a considerable number of capsules. Since most of our geriatric patients are on medication with Rauwolfia or pentylenetetrazole, or both, and receive regularly vitamin supplementation as is common in the management of elderly depressed patients with chronic degenerative vascular dis-

TABLE 1.—SERUM CHOLESTEROL LEVELS DURING CONTINUOUS TREATMENT FOR 10 WEEKS OR MORE WITH LARGE DOSES OF NIACIN SUPPLEMENTED WITH PYRIDOXINE IN A GROUP OF 20 PATIENTS WITH HYPERCHOLESTEROLEMIA

| NO. | CASE | SEX | AGE | DIAGNOSIS | FASTING SERUM CHOLESTEROL LEVEL | | | | | | | DOSAGE | SIDE EFFECTS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | INITIAL | WEEKS 2 | 4 | 6 | 8 | 10 | 12 | GM. PER DAY | FLUSHING | OTHER |
| 1 | B.C. | F | 69 | CVA, Hypertension, Arteriosclerotic cardiovascular disease. | 289 | 224 | 177 | 189 | 203 | 182 | | 3 | Mild | |
| 2 | B.S. | F | 79 | Hypertensive arteriosclerotic cardiovascular disease. | 329 | 325 | 283 | 303 | 254 | 285 | | 2 | Marked | |
| 3 | D.L. | F | 82 | Generalized atherosclerosis. | 378 | 198 | 236 | 179 | 187 | 165 | | 3 | Mild | |
| 4 | R.M. | F | 57 | Rheumatic heart disease, Atrial fibrillation, Cerebral embolus. | 306 | 221 | 265 | 227 | 241 | 215 | | 3 | Mild | Soft stool. |
| 5 | H.G. | M | 57 | CVA, Arteriosclerotic cardiovascular disease. | 347 | 273 | 280 | 307 | 288 | 156 | | 2 until 8th wk. 3 thereafter. | Minimal | |
| 6 | M.L. | M | 64 | Generalized atherosclerosis. | 346 | 231 | 244 | 300 | 297 | 222 | | 3 | Moderate | Soft stool. |
| 7 | M.G. | M | 46 | Multiple sclerosis. | 256 | 187 | 205 | 219 | 216 | 148 | | 3 | — | — |
| 8 | S.F. | M | 30 | Cerebral Palsy | 259 | 213 | 246 | 247 | 231 | 201 | | 3 | — | — |
| 9 | M.T. | M | 43 | Cerebral Palsy | 258 | 211 | — | — | 203 | 181 | | 3 | — | — |
| 10 | M.B. | F | 80 | Generalized Atherosclerosis. | 379 | — | 247 | 211 | 250 | 218 | | 3 | Minimal | Palpitations. |
| 11 | G.O. | F | 68 | Arteriosclerotic | 314 | 196 | 166 | 235 | 184 | 215 | | 3 | Minimal | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | G.O. | F | 68 | Arteriosclerotic Parkinsonism. | 314 | 196 | 166 | 235 | 184 | 215 | | 3 | Minimal | |
| 12 | M.Li. | M | 69 | CVA, Diabetes, Atherosclerotic cardiovascular disease. | 271 | 175 | 187 | 151 | 173 | 148 | 128 | 2 | — | — |
| 13 | I.P. | M | 75 | CVA, Hypertensive arteriosclerotic cardiovascular disease. | 240 | – | 136 | – | 85 | 118 | 125 | 2 | Moderate | |
| 14 | J.K. | M | 71 | CVA, Diabetes, Generalized atherosclerosis. | 433 | 380 | 423 | 318 | 365 | 268 | | 3 | — | — |
| 15 | H.A. | M | 72 | CVA, Generalized atherosclerosis. | 334 | 280 | 279 | 178 | 290 | 220 | | 3 | Marked | |
| 16 | F.F. | F | 52 | CVA, Hypertensive atherosclerotic cardiovascular disease. | 388 | 225 | – | 280 | 225 | 180 | | 3 | Moderate | |
| 17 | M.R. | M | 57 | Arteriosclerotic Parkinsonism. | 293 | 260 | – | – | 217 | 229 | | 3 | Minimal | Poor sleep |
| 18 | B.H. | F | 78 | Arteriosclerotic cardiovascular disease. Hypothyroid? | 444 | 281 | 296 | 295 | 291 | 263 | | 3 | Minimal | |
| 19 | R.H. | F | 68 | CVA, Generalized atherosis, Obesity, Hypothyroid. | 454 | 289 | – | 275 | 261 | 263 | | 3 | Minimal | Nausea, Occas. diarrhea |
| 20 | E.F. | F | 32 | Myxedema | 453 | – | 251 | 280 | 277 | 182 | | 3 | Mild | |
| | | | | AVERAGE — (TOTAL) Average of 16 patients on 3 gm. per day | 338 | 251 | 244 | 246 | 236 | 203 | | | | |
| | | | | | 346 | – | – | – | – | 209 | | | | |

cases, an attempt was made to combine these drugs with the nicotinic acid in one tablet. In an effort to increase the efficiency and cut down side effects of the medication, we added to the tablet a small dose of Vitamin $B_6$ and Vitamin C. Pyridoxine was added for the specific reason that it plays a role in the synthesis of unsaturated fatty acids (Sinclair[8] and Whitton and Holman[9]), and thus might potentiate the hypocholesterolemic effect of niacin. None of the other components appears to be implicated in cholesterol or fat metabolism. We thus arrived at a formula* which combined in each tablet:

| | | |
|---|---|---|
| Niacin | 330.0 | mg. |
| Pyridoxine HCl | 0.5 | mg. |
| Thiamine mononitrate | 1.0 | mg. |
| Riboflavin | 2.0 | mg. |
| Ascorbic acid | 20.0 | mg. |
| Cyanocobalamine ($B_{12}$ activ.) | .001 | mg. |
| Reserpine | .025 | mg. |
| Pentylenetetrazole | 20.0 | mg. |

DOSAGE. With the exception of the very first cases in which niacin alone was used, these tablets, designated as DC 710, were used. One tablet t.i.d. was administered during the first 2 or 3 days. The dosage was then increased to 2 tablets t.i.d. for the remainder of the first week and increased further to 3 tablets t.i.d. after the second week or later, as seen fit. Larger doses were neither used nor required. The tablets were given together with meals.

THE CONTROLS. During the treatment periods, the patients were kept as closely as possible under the same environmental conditions. This applied particularly to their nutrition. For the inpatients who represent the great majority of our group, this was easily achieved. The hospital diet is a rather constant one. All inpatients had been on a low fat diet consisting of 100 gm. protein, 50 gm. fat and 250 gm. carbohydrate and supplying about 2000 calories. Wherever hypocholesterolemic agents had been used previously, these were discontinued and a control period of sufficient length was provided to establish base levels of the cholesterolemia. The blind test was applied inasmuch as neither the patients nor the ward personnel were informed about the purpose of the medication, which was said to be a new vitamin preparation. Placebos, however, were not used. This appeared unnecessary for 2 reasons: 1) The expected marked objective changes in the serum cholesterol level did hardly require placebo-controls which are in place where subjective errors must be avoided. 2) The chronically ill institutionalized patient is not easily affected emotionally by a new drug. He usually has adjusted and resigned to his invalidism and harbors little hope for improvement. He reluctantly enters any new treatment trial, particularly since this usually involves not only the discomfort of ingesting drugs, but also repeated tests of blood or urine and other infringements of his already limited freedom.

Serum cholesterol determinations were done in all patients at frequent intervals, once weekly or once biweekly. Only the total serum cholesterol level was estimated, using the method by Zak, Dickenman, White, Burnett and Cherney[10].

A complete physical checkup was carried out at least weekly. All patients had complete blood counts at about 2-week intervals and weekly urinalyses. Serial liver function tests, blood sugar determinations, and other special examinations including EKG were done in selected patients or where warranted. The liver function tests included cephalin flocculation, thymol turbidity, and the total serum protein with the A/G ratio.

**Results.** A. CONTINUOUS PROLONGED MEDICATION WITH LARGE DOSES. The most impressive result was the regularity with which a hypocholesteremic effect of some degree could be observed in each of the patients under study. This occurred within the first 2 weeks. With continued therapy, the level usually decreased further but less precipitously and a plateau was reached gradually. Occasionally increases in the serum cholesterol level occurred, but these rarely reached the initial hypercholesterolemic values, and usually were soon followed by a new fall. Representative serum cholesterol curves are demonstrated in Fig. 1 which shows the course of Cases 1, 40, 18 and 41.

The pertinent data of the 20 patients on continuous medication for 10 weeks are shown in Table 1. It will be noted

*This formula was kindly prepared for us by Dr. B. J. Brent, Clinical Research Director of the Wampole Laboratories, Stamford, Conn. It has received the trade name Sin-Sterol.

TABLE 2.—SERUM CHOLESTEROL LEVELS AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH HYPERCHOLESTEROLEMIA UNDER TREATMENT WITH RELATIVELY SMALL DOSES OF NICOTINIC ACID

| | | | | | *Nicotinic Acid Medication* | | *Serum Cholesterol* | *SF 12–20* | *SF 20–100* |
|---|---|---|---|---|---|---|---|---|---|
| *No.* | *Case* | *Sex* | *Age* | *Diagnosis* | *Duration* | *Doses mg.* | *mg./100 ml.* | *mg./100 ml.* | *mg./100 ml.* |
| 1 | B. Te. | M | 44 | Xanthelasma | — | — | 270 | 60 | 110 |
| | | | | | 6 wks. | 200 | 210 | 45 | 87 |
| 2 | B. Nov. | F | 42 | Obesity, hypertension | — | — | 222 | 47 | 62 |
| | | | | | 6 wks. | 200 | 196 | 38 | 53 |
| 3 | A. Sch. | M | 53 | Coronary artery disease, obesity, old myocardial infarct. | — | — | 260 | 47 | 62 |
| | | | | | 8 wks. | 200 | 214 | 40 | 51 |
| 4 | A. Be. | M | 45 | Normal | — | — | 240 | — | — |
| | | | | | 4 wks. | 200 | 210 | — | — |
| 5 | T. Co. | M | 47 | Normal | — | — | 210 | — | — |
| | | | | | 4 wks. | 100 | 200 | — | — |
| 6 | M. Gov. | F | 52 | Diabetes mellitus (controlled) | — | — | 230 | — | — |
| | | | | | 6 wks. | 200 | 205 | — | — |
| 7 | H. Br. | M | 46 | Normal | — | — | 233 | — | — |
| | | | | | 4 wks. | 200 | 214 | — | — |

that 4 patients of this group were on only 2 gm. of nicotinic acid per day throughout this period, and that they too showed rather marked decreases of their serum cholesterol level. In 9 patients the total serum cholesterol fell to levels below 200 mg. per 100 ml., in 7 below 250 mg. and in only 4 the 10 week level was higher than 250 mg., but still significantly lower than the initial hypercholesterolemia. The decrease in the serum cholesterol occurred in the arteriosclerotic patients as well as in the hypothyroid and diabetic subjects. The table demonstrates, moreover, that the mean cholesterol decrease in the 16 patients receiving 3 gm. of niacin in the form of DC 710 for a period of 10 weeks was highly significant. It fell during this period from an initial mean of 345 mg. per 100 ml. to a mean of 209 mg., a drop

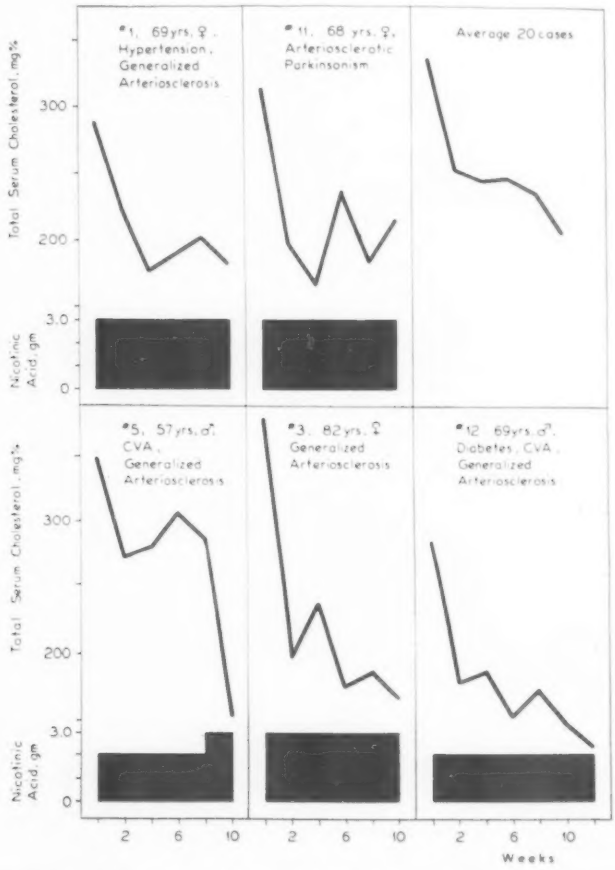


Fig. 1.—The course of the serum cholesterol level during continuous treatment with large doses of niacin supplemented with pyridoxine in 5 individual patients with hypercholesterolemia and the average fall of the serum cholesterol in the group of 20 patients under continuous treatment for 10 weeks or more.

of 137 mg. per 100 ml., or 39%. A reduction occurred in every one of the patients and the mean drop is highly significant ($p<.001$). The course of Case 51 is illustrative and of special interest:

**Case Report.** K.S. is a 57-year-old white man with idiopathic (familial) hypercholesterolemia and hyperlipemia, mild hyperglycemia and glycosuria and long standing complaints of precordial pain and intermittent claudication. His mother suffered from similar vascular symptoms and one brother died of coronary thrombosis at the age of 44. The patient is not obese, has a blood pressure within normal range and an electrocardiogram showing nothing more than left ventricular strain. His heart is not enlarged and there are no abnormal sounds. The pulsations in the popliteal arteries and in the dorsalis pedis were decreased bilaterally. Radiographically there was evidence of calcifications both in the aortic arch and the vessels of both lower extremities. The oscillometric readings and skin temperature over calves were decreased. His serum has been known for years to be milky and to have a total lipid level of more than 3000 mg. per 100 ml. with a cholesterol level of an average of 600 mg. per 100 ml. This level did not respond to estrogens or thyroid medication but decreased somewhat during a period of a diet entirely void of animal fats. Even then, however, the lowest level obtained was still 470 mg. per 100 ml. with the majority of the levels between 520 and 600, and no significant effect on the turbidity of the serum. During the past 5 months, the patient had been on a low fat diet without any medication and his serum cholesterol level had increased to values between 600 and 700 mg. per 100 ml. He felt tired, depressed and was unable to walk more than 2 blocks without pain in his calves. In addition, he had experienced a new, rather uncommon symptom of hyperalgesia in both hands. Immediately before starting the medication with the tablets DC 710, his serum cholesterol level was found to be as high as 1000 mg. and his total serum lipids were 12,400 mg. per ml. He was given 3 × 2 tablets daily for the first 2 weeks, at the end of which the serum cholesterol level had decreased to 520 mg. per 100 ml., with continued medication of 3 × 3 tablets daily for 3 weeks a level of 344 mg. per 100 ml. was obtained. His serum had lost most of its turbidity and its total lipid content was 1200 mg. per 100 ml. Moreover, within a few days on DC 710, the patient had lost the painful sensation in his hands and did not experience leg cramps when walking 5 or 6 blocks. He felt less tired and more ambitious. After another week a level of 256 mg. per 100 ml. serum cholesterol was obtained. This level has been maintained in spite of the fact that the fat intake was increased from 30 gm. to 60 gm. daily and the medication decreased after the 10th week to 4 tablets daily. The side effects of the medication (see below) which he noted were not only the rather common and unpleasant flushing, but also the occurrence of more voluminous soft and foul-smelling feces. It might be mentioned, however, that the glycosuria and hyperglycemia increased slightly but was controlled with 1 gm. Tolbutamide daily.

Similar gratifying results were obtained in each patient who has continued the medication for more than one week.

B. INTERRUPTED MEDICATION WITH LARGE DOSES. As soon as the drug was withheld, the initial hypercholesterolemia returned. Within 2 or 3 days, a marked rise of the serum cholesterol occurred but a new fall could be obtained as early as 48 hours after resumption of the effective dose. This rapid rise was as striking as the sustained decrease of the levels as long as medication was adequate. Illustrative curves are shown in Fig. 2.

**Case Reports.** Case 25. A 57-year-old man with arteriosclerotic Parkinsonism refused the medication after one week because of severe flushing, irritability and inability to sleep. His initial serum cholesterol level was 293 mg. per 100 ml.; at the last day of medication with 3 × 3 tablets it had decreased to 234 mg. per 100 ml. Another estimation done 48 hours later, was 317 mg. per 100 ml. Two weeks later the patient was willing to resume the medication. This time the initial level was 302 and after 2 days of medication of 3 × 3 tablets it had then decreased to 229 mg. per 100 ml. and 2 weeks later to 206 mg. per 100 ml.

Case 49. A 32-year-old woman with hypercholesterolemia had had an excellent response to 3 × 3 tablets daily; her serum

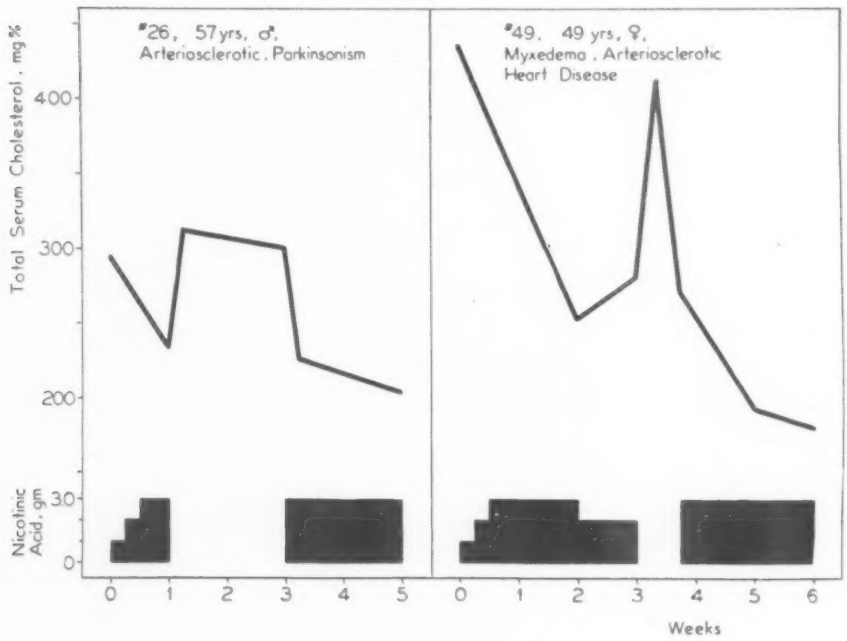


Fig. 2.—The effect of interrupted treatment with large doses of niacin supplemented with pyridoxine on the serum cholesterol level in 2 patients with hypercholesterolemia.

TABLE 3.—LIVER FUNCTION TESTS AND FASTING SUGAR LEVELS BEFORE AND DURING TREATMENT WITH NIACIN IN 12 PATIENTS. THE CASE NUMBERS REFER TO THE CASES IN TABLE 1

| *Case No.* | *A/G Ratio* | | *Ceph. Floc.* | | *Thymol Turbidity* | | *FBS* | |
|---|---|---|---|---|---|---|---|---|
| | *Before* | *During* | *Before* | *During* | *Before* | *During* | *Before* | *During* |
| 1* | 3.44/4.25 | 3.2/4.2 | 1+ | 2+ | — | — | 98 | 86 |
| 3† | 3.76/3.30 | 3.50/3.30 | — | — | 2.8 | 1.59 | 102 | 95 |
| 5* | 4.07/3.49 | 3.65/3.45 | — | 1+ | — | — | 89 | 93 |
| 7** | 3.84/3.61 | 4.39/4.10 | — | — | — | — | 84 | 104 |
| 8** | 4.39/3.00 | 4.50/3.10 | — | — | — | — | 77 | 82 |
| 9** | 5.0/2.24 | 4.60/2.50 | — | — | — | — | 95 | 68 |
| 10‡ | 3.76/3.88 | 3.5/3.5 | 3+ | 2+ | 2.3 | 3.3 | 112 | 95 |
| 11‡ | 3.64/3.86 | 3.9/3.0 | — | — | — | 2.3 | 79 | 70 |
| 15** | 4.64/2.10 | 4.0/3.3 | 2+ | — | 0.9 | 0.23 | 106 | 83 |
| 16** | 3.65/3.84 | 3.70/3.81 | 2+ | 2+ | 3.4 | 3.9 | 110 | 89 |
| 18* | 3.40/3.80 | 3.77/3.6 | 1+ | — | 2.8 | 3.4 | 104 | 90 |
| 20** | 4.30/3.03 | 4.05/2.81 | — | — | 2.3 | 1.76 | 88 | 68 |

*Indicates 2nd test after 4 weeks of treatment.

†Indicates 2nd test after 6 weeks of treatment.

‡Indicates 2nd test after 8 weeks of treatment.

**Indicates 2nd test after 10 weeks of treatment.

The fasting blood sugar levels represent averages of 3 to 5 determinations done at intervals.

cholesterol level had fallen within 2 weeks from 435 to 251 mg. per 100 ml. The dose was then decreased to 3 × 2 tablets, whereupon only a small increase occurred to 280 mg. per 100 ml. At this time medication was withheld for 2 days with a subsequent rise to 410 mg. per 100 ml. Resumption of medication with the initial dose brought within 48 hours a fall to 270 mg. per 100 ml. which dropped further to 192 and 180 mg. per 100 ml. at subsequent weekly intervals.

C. STUDIES WITH SMALLER DOSES OF NIACIN. In view of Altschul's observation of some hypocholesterolemic effects with 1 gm. per day of niacin and our own finding that a few patients could be treated effectively with approximately 2 gm. per day, we attempted an evaluation of still lower doses. Patients received as little as 25 mg. niacin q.i.d. No response of the serum cholesterol could be noted. With increase of the dosage to 50 mg. q.i.d., however, small and sustained drops were obtained. Representative data for this group is shown in Table 3. The flotation fractions SF 12–20 and 20–100 of the beta-lipoproteins were estimated in these patients and are listed in the table for the 3 patients in whom a hypocholesterolemic effect appeared. Their changes are not significant.

SIDE EFFECTS. The most disturbing and most common side effect was flushing and the feeling of warmth and lightheadedness. Most every patient experienced it, at least during the first week of medication. It occurred with the small doses as well as with the largest. The addition of reserpine and pentylenetetrazole to the medication seems to have brought some improvement, but a large group of the patients continued to be bothered by these symptoms to some degree. The flushing usually persists for a half to one hour. Most patients got used to this annoying feeling or noted a subsidence with continued medication. Fifteen patients, however, refused continuation of the medication after one week.

Next common was the feeling of nausea and occasional vomiting. This occurred particularly when the medication was taken on an empty stomach. It could be prevented by offering the tablets together with the meals.

A number of patients observed that their feces had become more voluminous and foul smelling. Four patients complained in addition of persistent diarrhea. The feces had the shiny appearance of fatty stools. The diarrhea could easily be controlled with kaopek.

Other patients, 3 in the whole group of 53, noted transitory episodes of palpitation and a few complained about difficulties in falling asleep.

Outside of the flushing episodes, there was no impairment of the general well being. Many patients indicated that their appetite had increased and they showed in general more interest and activity. With regard to the cardiovascular system, there were no noticeable persistent changes in the peripheral circulation, no changes in the blood pressure or the electrocardiogram. It is noteworthy that none of the patients with previous coronary disease experienced any recurrence of precordial pain or angina.

Serial liver function tests were done in 12 of the 20 patients treated for 10 weeks or longer. The tests were done before treatment and either after 4, 6, 8, or 10 weeks of medication. No significant changes were noted in any of the tested parameters, at any interval. The data for the individual patients are given in Table 3. This table demonstrates also that the fasting blood sugar level was not significantly influenced by the medication. The values during treatment represent the averages of 3 or more determinations done at various intervals during the treatment period. In a few patients

the diurnal blood sugar level was followed and no significant alterations were observed.

As is to be expected in a prolonged study of elderly and seriously disabled patients, there occurred a few fatalities which most likely are not related to the drug. A 96-year-old woman with advanced atherosclerosis died of bronchopneumonia; a 60-year-old woman with atherosclerosis developed mesenteric thrombosis and died with peritonitis.

**Discussion.** Our observations leave no doubt that niacin in appropriate dosage, particularly in combination with pyridoxine, is a potent serum-cholesterol lowering agent. Our results during a 10 weeks' period with a dosage containing 3 gm. of niacin and 3 mg. pyridoxine per day were somewhat superior to those obtained by Parsons *et al.*[5,6]. The mean starting serum cholesterol level in our series of 20 patients decreased from 338 mg. to 203 mg. per 100 ml., and none of the patients failed to show a response. The two series are, of course, not identical in every respect, and, therefore, not easily comparable. Thirteen of our patients observed for a 10 weeks' period had an initial serum cholesterol level of 270 mg. per 100 ml., or higher, as the 23 patients observed by Parsons and Flinn[6] over 12 weeks. While in this latter series 9 showed no significant response, 6 had a reduction from the initial level of 40 mg. per 100 ml. or more, and 8 had a final level below 250 mg., all 13 of our series showed a significant response with a fall to or below 250 mg. per 100 ml. in 10 of them. The Chi-square test shows that this difference is statistically significant ($P = .02$).

The efficacy of this medication seems to be greater than that of any other known means of lowering the serum cholesterol level. It exerts its effect in the various types of hypercholesterolemia, the familial or idiopathic as well as the acquired, the hypercholesterolemia of uncontrolled diabetes and of myxedema.

The magnitude of the effect is so great that it cannot be explained by interference with the dietary or exogenous cholesterol alone. Relaxation of dietary restriction, as in Case 51, did not lead to a resurgence of the serum cholesterol. It is likely that the endogenous cholesterol metabolism is affected as well.

The absence of serious side effects makes niacin even in high doses an acceptable clinical drug though the unpleasant signs and symptoms of vasodilatation as flushing and feeling of heat may be a deterrent to many patients. Our attempts to combine it with an ataractic and tranquilizer have brought some, but not sufficient improvement with regard to the flushing effect. The addition of pyridoxine seems to have lowered the effective dose. It will be worth while to investigate whether other combinations or modifications can potentiate the serum cholesterol lowering effect of niacin and permit elimination of the hyperemia.

The possibility of liver impairment by prolonged medication of large doses of niacin has to be considered. Niacin, at least to a large extent, is transformed to nicotinamide and then methylated in the liver. The prime methyl-donor is methionine. Thus, this lipotropic factor may be deviated from its physiological function. Fatty livers have indeed been demonstrated in the animal experiment after administration of unusually large doses of nicotinamide (Krehl[3]). No such observations have been reported in man. No liver damage was demonstrated by the liver function tests in our patients. It appears, therefore, that a normal protein intake of 100 gm. per day, as

provided in our series, will give adequate protection against this danger.

Niacin has been reported recently to inhibit enzymatic insulin degradation and thus to induce hypoglycemia (Mirsky, Perisutti and Jinks[4]). Although we did not note in our patients any effect on the fasting blood sugar level, further studies are under way employing both the glucose tolerance and the insulin sensitivity tests. These will be reported later.

The mechanism of the action on cholesterol metabolism is as yet entirely unknown. The hypocholesterolemic effects can hardly be considered as the result of substitution therapy since the effective doses are far in excess of those required to correct nicotinic acid deficiency, for example, in pellagra. One must think of a pharmacological effect. This is reminiscent of Jolliffe's[2] earlier observation of the beneficial role of large doses of nicotinic acid in the treatment of alcoholic encephalopathies. Altschul[1] has suggested that niacin as part of the respiratory enzyme system might increase tissue oxidation and thus lead to faster oxidation of cholesterol, and that no reabsorbable oxycholesterols may be formed and excreted. An interference with the reabsorption or absorption of cholesterol from the gut would indeed explain the rapid development and the equally fast disappearance of the effect. One would have to assume that the endogenous cholesterol reaching the gut through the biliary tract as well as the dietary cholesterol are prevented from entering the intestinal mucosa. Our observation of the occurrence of voluminous foul-smelling and shiny stool seems to support this concept. These stools, however, occurred in only a few patients. The changes in the beta-lipoprotein fractions under niacin medication as reported from the Mayo Clinic, and our clinical observation in the case of familial idiopathic hyperlipemia and hypercholesterolemia (Case 51) of the clearing of the hyperlipemic serum may suggest that niacin has some effect on the clearing factor of the blood and on lipid transport. These problems deserve further investigation. Preliminary data on patients with biliary drainage seem to indicate that the niacin hypocholesterolemia is accompanied by an increased biliary bile flow[7].

Equally important will be clinical and pathological studies on the effect of niacin medication on the development and the course of degenerative vascular diseases. Evident as the relationship between hypercholesterolemia and atherosclerosis is, it is far from clear what role the hypercholesterolemia plays in the chain of causative factors.

**Summary.** In a group of 53 patients with hypercholesterolemia a marked and sustained lowering of the serum cholesterol level was obtained by medication of a combination of niacin and pyridoxine. The effective daily dose ranged between 2 and 3 gm. niacin and 2 to 3 gm. pyridoxine. The addition to the medication of Vitamin C, Rauwolfia and metrazole appears to have decreased the incidence and severity of the side effects of niacin, particularly the skin hyperemia and the feeling of heat and flushing. In 20 patients who were treated with this formula for more than 10 weeks, the serum cholesterol level fell from an initial average of 338 mg. per 100 ml. to an average of 203 mg. per 100 ml. after 10 weeks. Hypercholesterolemias of various types showed significant responses. Discontinuation of the drug was followed immediately by restoration of the pre-existent hypercholesterolemia.

ACKNOWLEDGMENT The authors are indebted to Mr. A. Saifer, of the Department of Laboratories of the Jewish Chronic Disease Hospital, for advice and cooperation, and to Doctors Theodore T. Cohn and I. J. Greenblatt of the Messinger Research Laboratories for supplying the data on lipid fractionation by ultracentrifugation.

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## SUMMARIO IN INTERLINGUA

### Marcate E Sustenite Grados De Reduction Del Cholesterol Del Sanguine, Effectuate Per Medication Con Niacina E Pyridoxina

In un gruppo de 53 patientes con hypercholesterolemia, marcate e sustenite reductiones del nivello seral de cholesterol esseva effectuate per un medication combinate con niacina e pyridoxina. Le efficace dose diurne variava inter 2 e 3 g de niacina e inter 2 e 3 g de pyridoxina. Le addition, a iste medication, de vitamina C, Rauwolfia, e metrazol pare haber reducite le incidentia e le severitate del effectos lateral de niacina, specialmente le hyperemia del pelle e le sensation de calor e de rubor. In 20 patientes, qui recipeva iste tractamento durante plus que 10 septimanas, le nivello seral de cholesterol descendeva ab un nivello medie initial de 338 mg per 100 ml a un nivello medie al fin de 10 septimanas de 203 mg per 100 ml. Hypercholesterolemias de varie typos monstrava responsas significative. Le discontinuation del droga esseva sequite immediatemente per le restauration del pre-existente hypercholesterolemia.

# CLINICAL STUDIES ON THE CONTROL OF SODIUM AND WATER METABOLISM IN PANHYPOPITUITARISM

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The control of sodium and water metabolism in the absence of anterior pituitary function is still not completely understood. The syndrome of hyponatremia in panhypopituitarism has been well described in 1955 by Wynn and Garrod[20] who attributed it to water retention. Others have claimed a defective mechanism for the renal conservation of sodium due to the lack of an increased adrenocortical secretion of aldosterone in response to sodium deprivation in the absence of adequate anterior pituitary function (Hernando *et al.*[8]). Our results, presented in the present paper, confirm those of Venning *et al.*[19] and Maclean *et al.*[13] who found a normal response to sodium restriction in panhypopituitarism and after hypophysectomy, and who attributed the hyponatremia found in some untreated patients to water retention.

The inadequate excretion of a water load present in patients with untreated Addison's disease and panhypopituitarism can be corrected by cortisone. Cortisone and other 17-hydroxycorticoids have been shown to have a diuretic effect in a number of patients with edema from various causes. This effect of 17-hydroxycorticoids on the renal handling of water may be due to a direct action on the kidney (Raisz *et al.*[16]) or through a certain antagonistic action to ADH (Dingman and Despointes[5] and Ikkos, Luft and Olivecrona[9]). Our results presented in the present paper demonstrate that 17-hydroxycorticoids do not inhibit anti-diuretic hormone (ADH) and do not prevent or modify the action of ADH on the kidney.

**Case Report.** Mrs. P.S. is a 62-year-old white widow admitted to the gynecological service of Notre Dame hospital on July 22, 1957, for a grade II cystocele and a cervical polyp. She had had 7 normal pregnancies and 2 miscarriages without any important hemorrhage. She remembers having hot flashes during the menopause at the age of 38.

On October 27, 1952, she was admitted to the surgical service of Notre Dame Hospital complaining of anorexia, nausea and vomiting. At that time a simple roentgenogram of the abdomen and complete Roentgen studies of the gastrointestinal tract were negative. There were a few leukocytes in the urinary sediment but repeated cultures were negative. The hematocrit was 35%, the white blood cell count showed a total of 6,000 per c.mm., with 53% neutrophils, 36% lymphocytes, 3% monocytes and 8% eosinophils. Blood glucose and urea were within normal limits. Roentgenograms of the chest disclosed a small fibrotic area at the left apex, probably a healed tuberculous lesion. Seven days after admission, the patient, who was still vomiting, became weaker, disoriented and finally semiconscious. Complete neurological examination was negative as were the fundi and roentgenograms of the skull. She improved rapidly under therapy with 0.9% saline and 10% glucose solutions intravenously. Five days later, she was transferred to the medical service from which she was discharged on November 15, 1952.

From the time of discharge in 1952 until re-admission in 1957 she complained constantly of weakness and fatigability with

anorexia, nausea and occasionally vomiting. She also complained of chronic constipation, nervousness and insomnia. She had lost approximately 9 kilograms, during those five years.

On physical examination the patient was pale and very thin. Her height was 1.46 meter and she weighed 39.1 kg. The temperature was 36.7° C., pulse 80, respirations 14 and blood pressure 135/80. Complete absence of axillary hair and very scanty pubic hair were noted. Pelvic examination disclosed a grade II cystocele and a small cervical polyp. The uterus was noted to be very small.

Complete urinalysis, blood sugar and urea were normal. There were 4,000,000 red blood cells per c.mm. and 10.5 gm.% hemoglobin. The white blood cells numbered 7,250 per c.mm. with 41% neutrophils, 46% lymphocytes, 5% monocytes and 8% eosinophils. The following day, the patient was noted to be very weak during later afternoon and next morning was found to be semiconscious in bed. At that time, the rectal temperature was 38.9° C. and rose to 39.4° C. in the afternoon. The blood pressure was 80/60. The patient was very pale and appeared slightly dehydrated. The skin was fine and dry. Complete neurological and fundoscopic examinations were negative. The cardiac rate was irregular at 120 beats per minute and the electrocardiogram confirmed the clinical impression of atrial fibrillation but failed to show any other abnormality.

The following laboratory and Roentgen-ray studies were done on an emergency basis: blood urea 21 mg. per 100 ml., blood sugar 100 mg. per 100 ml., hematocrit 42%. Total white blood cells were 18,500 with 64% neutrophils, 33% lymphocytes, 3% monocytes and 0 eosinophils. The serum sodium was 124 mEq. per liter, serum potassium 5.3 mEq. per liter, serum chloride 84 mEq. per liter and $CO_2$ content 20 mmol. per liter. Roentgenograms of the skull were negative and an A.P. film of the chest was also negative except for the healed tuberculous lesion previously described at the right apex. Two blood cultures and one urine culture did not show any growth in the following days. The patient was then transferred to the medical service. She received 600,000 units of S.R. penicillin and 0.5 gm. of streptomycin intramuscularly daily. During the first 24 hours, she was also given intravenously 500 ml. of 3% sodium chloride solution followed by 2 liters of 0.9% sodium chloride solution. A few hours later the atrial fibrillation disappeared and an electrocardiogram showed a normal sinus rhythm. The next day she was completely conscious but still weak and her serum sodium had risen to 136 mEq. per liter. On the fourth day, she felt much better, could eat and drink and could get out of bed for short periods of time. The temperature was 39.7° C. and the blood pressure 120/80.

From July 30 to August 9 she received a total of 300 mEq. of sodium per day while the following laboratory studies were obtained. There were 3,000,000 red blood cells per c.mm. with 8.4 gm.% hemoglobin. The white blood cells numbered 6,000 per c. mm. with 49% neutrophils, 38% lymphocytes, 5% monocytes and 8% eosinophils. Serum sodium was 142 mEq. per liter and serum potassium 4.9 mEq. per liter. Total cholesterol was 160 mg. per 100 ml., the basal metabolic rate was −14, serum protein-bound iodine 3 mcg.% and the radioiodine uptake was 11% after 24 hours. F.S.H. assay on a 24-hour urine collection was negative at 6.6 mouse units. Urinary 17-ketosteroids and 17-hydroxycorticoids were measured during a control period and during the standard 2-day intravenous ACTH test. The following values were found. On the 2 control days, 17-ketosteroids were 0.4 and 0.5 mg. per 24 hours and rose to 1.8 on the first day and to 9.1 mg. on the second day of ACTH. The 17-hydroxycorticoids were 0.4 and 3.4 mg. on the 2 control days and increased to 4.4 and 5.2 mg. under ACTH stimulation. A TSH test was not done because the patient had a cholecystogram after the initial PBI and RAI uptake. On the basis of the clinical picture and laboratory studies, a diagnosis of panhypopituitarism of undetermined etiology was made.

At the end of the first study on a low Na diet, while still untreated, the patient almost went into shock with a urinary infection due to *E. coli*. She was treated immediately with chloramphenicol, intravenous hydrocortisone and later with oral cortisone. Five days later she was well and a urine culture was negative. Starting August 23, she was maintained on cortisone 25 mg. and thyroid extract 60 mg. per day. Because of urinary infection, the following renal function studies were done. Phenolsulfonphthalein excretion was 40% at 15 minutes and 80% at 2 hours. The urinary sediment was normal. Intravenous pyelograms showed a slightly delayed excretion from the left kidney and retrograde pyelograms were completely normal. The Na and K concentrations in the urine from each kidney were almost the same. She responded normally to Diamox and the urine became

TABLE 1.—EFFECT OF DIETARY SODIUM RESTRICTION IN UNTREATED PANHYPOPITUITARISM

PS. Q 624 HND 57-08237

| DATE AUG. 57 | Rx | WEIGHT KG | NA INTAKE mEq | NA URINE mEq | SERUM mEq/L | |
|---|---|---|---|---|---|---|
| | | | | | NA | K |
| 7 | 0 | 41.4 | ≈300 | 240 | 142 | 4.9 |
| 9 | 0 | 40.5 | 22 | | | |
| 15 | 0 | 40.5 | 22 | 75 | | |
| 16 | 0 | 41.0 | 22 | 80 | 120 | 5.5 |
| 19 | DOCA 10 mg | — | ≈350 | | 120 | 4.3 |
| 20 | DOCA 10 E 100 mg | — | ≈350 | | | |
| 21 | DOCA 10 E 100 mg | — | ≈350 | | 136 | 5.0 |
| 22 | DOCA 10 E 25 mg | — | ≈300 | | 145 | 4.4 |
| 27 | E 25 mg | 43.6 | ≈300 | 232 | 140 | 4.3 |

acidified under an ammonium chloride load. Total plasma proteins were 6.1 gm.% and the electrophoretic pattern was normal. She had no Bromsulphalein retention after 45 minutes. Two months after 60 mg. of thyroid extract daily, her basal metabolic rate had risen from −14 to + 7. She was discharged on November 22, 1957, on cortisone 12.5 mg. and thyroid extract 90 mg. orally, daily. Her weight which was 39.1 kg. on admission was 47.5 kg. on discharge and she felt very well.

**Methods.** The patient was studied on the medical service between July 25 and November 22, 1957.

The constant diet that she received for 17 days was calculated from standard reference tables (Bridge's Food and Beverage Analysis by M. R. Matice, ed. 1955).

Urinary 17-hydroxycorticosteroids were measured by the method of Glenn and Nelson[7] and the urinary 17-ketosteroids by the Method of Callow, Callow and Emmens[8]. Urinary aldosterone determinations were done according to the technique of Nowaczinski, Koiw and Genest[15].

**Results.** 1. EFFECT OF SODIUM RESTRICTION IN UNTREATED PANHYPOPITUITARISM. Table 1 shows the results of the first experiment which was done while the patient was not receiving any medication. Only a sodium balance was

done; calories, water and potassium balances were not controlled. During the control period of 7 days the patient received 300 mEq. of sodium per day. During that period, her weight remained constant at 41.5 kg., the serum electrolytes were within normal limits, and urinary sodium was slightly below intake. On August 9, the sodium intake was reduced to 22 mEq. per day. On the seventh day of the low sodium diet, she was still excreting 80 mEq. of sodium in the urine and the serum sodium had fallen to 120 mEq. per liter. The patient felt very weak and the experiment had to be stopped. We had succeeded in producing experimentally by sodium restriction a hyponatremia similar to the one she had presented on admission. However, the pathogenesis of the hyponatremia was still imprecise. Although she was still in a negative sodium balance at the end of the sodium restriction period, she had lost only 0.4 kg. of weight which suggested that a certain degree of water retention was contributing to the hyponatremia.

2. EFFECT OF SODIUM RESTRICTION IN PANHYPOPITUITARISM TREATED WITH PREDNISONE. This next experiment is shown in Fig. 1. During the whole study the patient received a constant diet of 30 calories per kg. for a daily total of 1200 calories, containing 80 mEq. of potassium and 11 mEq. of sodium. The daily total water intake was 1500 ml. During the control period of 4 days she received a daily supplement of 204 mEq. of sodium as sodium chloride. Throughout the control and experimental periods she was maintained on 5 mg. of prednisone and 60 mg. of thyroid extract daily. During the control period, the patient was in metabolic balance. Her weight remained constant at 38.5 kg., urinary sodium output corresponded to her intake, serum electrolytes were within normal limits, and the urinary aldosterone excretion was 12 mcg. per day which is at the higher limit of normal by the method used. During the experimental period, sodium intake was abruptly reduced to 11 mEq. per day, everything else remaining constant, and it can be seen (Fig. 1) that sodium balance was achieved on the fourth day; she lost approximately one kilogram, serum electrolytes remained within normal limits, and the urinary excretion of aldosterone rose to 25 mcg. per day.

From these 2 experiments it seems justified to conclude that the presence of an adequate amount of circulating 17-hydroxycorticoids enables this patient with panhypopituitarism to adapt herself normally to sodium restriction and to show the expected rise in urinary aldosterone excretion. As a corollary, it seems justifiable to postulate that the hyponatremia, which was observed when the patient was subjected to sodium restriction, while she was untreated, was due to the fact that she lost less water than sodium in the urine. It is conceivable that moderate and chronic primary water retention can produce a slight increase in extracellular fluid (ECF) volume which, in turn, can raise the glomerular filtration rate (GFR) (Leaf *et al.*[10]) and inhibit aldosterone production (Beck *et al.*[2]) resulting in an increased renal loss of sodium. This could explain the observed negative sodium balance while our patient, untreated, was put on sodium restriction, and it could also account for the absence of an increase in body weight, or for the presence of a slight fall, similar to the one that we observed in our patient (0.4 kg.).

3. PANHYPOPITUITARISM: THE EFFECT OF PREDNISONE ON THE RESPONSE TO A WATER LOAD. The water load was done using the standard dose of 20 ml. per kg. by mouth taken during ½ hour.

Urinary volume and specific gravity were measured every hour for 5 hours. The patient was still on a constant diet containing 70 gm. of protein and 215 mEq. of sodium. The experiments were done with the patient in the supine position. The results are shown in Fig. 2. Without prednisone, the patient excreted 22% of the water load in 5 hours while on 20 mg. of prednisone, she excreted 82% of the same water load during the same period of time. This experiment confirms the previous observation (Wynn and Garrod[20]) that the water retention which may be found in untreated panhypopituitarism can be attributed to a decreased adrenocortical secretion of 17-hydroxycorticoids. This experiment brings out again the possible inter-relations between 17-hydroxycorticoids, anti-diuretic hormone (ADH), and the renal control of water metabolism.

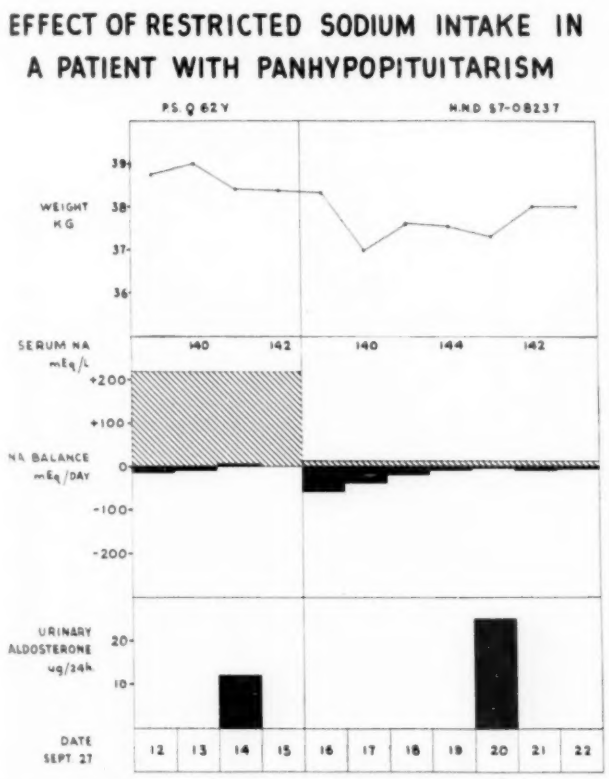


Fig. 1.—Effect of sodium restriction in panhypopituitarism treated with prednisone (5 mg. per day).

4. PANHYPOPITUITARISM: RESPONSE TO ADH DURING MAXIMAL WATER DIURESIS; EFFECT OF 2 DOSE LEVELS OF CORTISONE. To eliminate endogenous ADH secretion, maximal water diuresis was produced in the following manner. The patient was in the supine position during the whole experiment. Diuresis was induced by the standard dose of

20 ml. per kg. taken by mouth in ½ hour. Afterwards the patient drank an amount of water equivalent to the urinary volume of the preceding 15 minutes until maximal diuresis was obtained and maintained for 45 minutes. At that time, 5 milliunits of aqueous Pitressin, which was the smallest amount of Pitressin to elicit a response in this patient, were injected rapidly intravenously and the urine volumes were followed for another 45 minutes. One experiment was done with 25 mg. of cortisone and the other experiment was done with 100 mg. of cortisone. The results are shown in Fig. 3. It can be seen that the response was exactly the same in both instances. This experiment suggests that cortisone does not inhibit ADH and does not have an antagonistic action to ADH on the kidney.

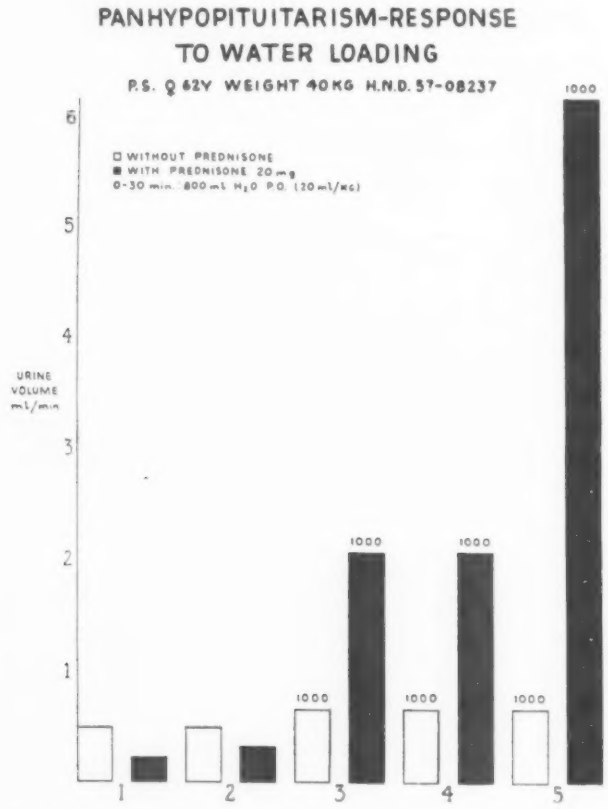


Fig. 2.—Panhypopitiuitarism: the effect of prednisone on the response to a water load.

**Discussion.** The dissociation existing in the control of adrenocortical secretion of glucocorticoids and androgens on one side, and of aldosterone on the other, has been well presented and discussed by Liddle, Duncan and Bartter[11]. That the control of aldosterone secretion is at least relatively independent of anterior pituitary function seems to be universally admitted. Normal levels of urinary aldosterone have been found in hypophysectomized

patients and in patients with panhypopituitarism (Luetscher and Axelrad[12] and Venning, Dyrenfurth and Beck[18]). No increase in the level of urinary aldosterone excretion under ACTH stimulation has been found in patients with panhypopituitarism (Liddle, Dunthe influence of cortisone (Venning, Dyrenfurth and Beck[18]) prednisone (Hernando *et al.*[8]) or 9 alpha-fluorohydrocortisone (Hernando *et al.*[8]) in normal subjects and patients with panhypopituitarism. Sodium deprivation is a most potent stimulus to

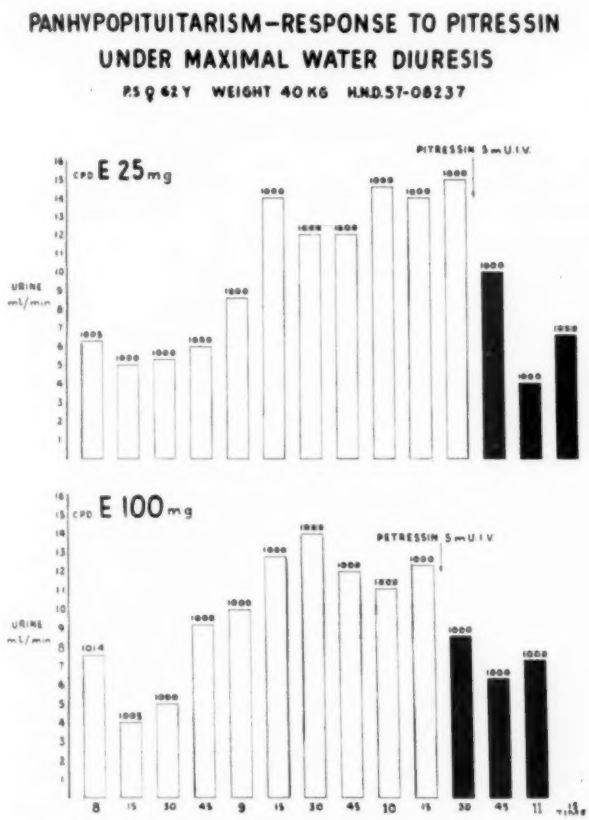


Fig. 3.—Panhypopituitarism: response to ADH during maximal water diuresis; effects of 2 dose levels of cortisone.

can and Bartter[11], Venning, Dyrenfurth and Beck[18]). In normal subjects under ACTH stimulation, irregular and variable increases in urinary aldosterone excretion have been found which are not comparable to the increases noted on sodium restriction (Hernando *et al.*[8]). No decrease in urinary aldosterone excretion has been found under aldosterone secretion in normal subjects and is supposed to act through a decrease in ECF volume or of one of its constituents (Bartter *et al.*[1]). Hypophysectomized patients on maintenance therapy with cortisone and thyroid extract adapt themselves normally to the stimulus of sodium restriction and show a rise in urinary

aldosterone excretion comparable to that found in normal subjects (Maclean *et al.*[13] and Muller, Riondel and Manning[14]). In panhypopituitarism on maintenance therapy with cortisone and thyroid extract, Hernando *et al.* described a female patient who adapted herself very sluggishly to sodium restriction and who failed to show a rise in urinary aldosterone excretion[8]. Our present patient with panhypopituitarism, on maintenance therapy with cortisone and thyroid extract, just as the one described by Venning *et al.*[19], adapted herself normally to sodium restriction and showed an increased urinary excretion of aldosterone comparable to the one found in normal subjects. From our experiments it seems justified to conclude that the presence of an adequate amount of circulating 17-hydroxycorticoids enables the patient with panhypopituitarism to adapt normally to sodium restriction and to show the expected rise in urinary aldosterone excretion. The hyponatremia which may be observed in untreated panhypopituitarism under sodium restriction can be attributed to the fact that there is less water than sodium lost in the urine. Moderate and chronic water retention, due to the decreased adrenocortical production of 17-hydroxycorticoids, can produce a slight increase in extracellular fluid volume which, in turn, can raise the GFR (Leaf *et al.*[10]) and inhibit the secretion of aldosterone (Beck *et al.*[2]), resulting in an increased renal loss of sodium. This mechanism would try to limit the increase in ECF volume and in body weight during water retention and, at the same time, would tend to aggravate the hyponatremia.

This brings us to the possible interrelations between 17-hydroxycorticoids, ADH and the renal handling of water. Patients with untreated Addison's disease show a delay in the elimination of a water load which may be corrected by cortisone. Patients with untreated panhypopituitarism also show the same delay in the elimination of a water load which may be corrected by treatment with ACTH (Chalmers and Lewis[4]) or cortisone, as illustrated in our patient. It is also often possible to elicit a diuresis in edematous patients with 17-hydroxycorticoids. It seems clear that there is a definite influence of 17-hydroxycorticoids on the renal control of water balance. From our studies it seems that 17-hydroxycorticoids do not inhibit ADH and do not have a competitive action antagonistic to ADH on the kidney. The possibility that 17-hydroxycorticoids may diminish the secretion of ADH, as suggested by Dingman and Despointes[5], cannot be considered at the moment because of the lack of a reliable method to measure ADH. However, it seems certain that 17-hydroxycorticoids may increase the renal elimination of water by a direct action on the kidney, without any relation to ADH. This direct renal action of 17-hydroxycorticoids may be due either to an increase in GFR (Skillern, Corcoran and Scherbel[17]), or to a decrease in the distal tubular reabsorption of solute-free water (Garrod, Davies and Cahill[6], Raisz *et al.*[16]), or to a combination of both mechanisms.

**Summary.** 1. A case of panhypopituitarism with hyponatremia is described.

2. From our studies, it is postulated that the hyponatremia, which may be produced by dietary sodium restriction in patients with untreated panhypopituitarism, is due primarily to water retention resulting from a decreased secretion of 17-hydroxycorticosteroids.

3. It is also suggested that 17-hydroxycorticosteroids may increase the renal excretion of water directly, without any antagonistic action to ADH.

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## SUMMARIO IN INTERLINGUA

### Studios Clinic Relative Al Regulation Del Metabolismo De Natrium E Aqua In Panhypopituitarismo

1. Es describite un caso de panhypopituitarismo con hyponatremia.

2. Es postulate, super le base de nostre studios, que le hyponatremia que pote esser producite per restriction dietari de natrium in patientes con non-tractate panhypopituitarismo, resulta primarimente ab le retention de aqua que, de su parte, es le effecto de un reducite secretion de 17-hydroxycorticosteroides.

3. Es etiam suggerite le possibilitate que 17-hydroxycorticosteroides augmenta le excretion renal de aqua directemente, sin ulle action antagonistic contra hormon anti-diuretic.

# THE DEGREE OF UNSATURATION OF PLASMA LIPID FRACTIONS IN CORONARY ARTERY DISEASE*

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Most investigators believe that a disorder of cholesterol metabolism is of great importance in the etiology of atherosclerosis. Reasons for this are: 1) the high cholesterol content of atherosclerotic plaques, 2) the production of atherosclerotic-like lesions in various species by feeding cholesterol, 3) the increased incidence of atherosclerosis in disorders of fat metabolism associated with high blood cholesterol levels, and 4) the elevated blood cholesterol and lipoprotein averages in large groups of atherosclerotic people. However, neither the blood cholesterol nor the lipoprotein level is of predictive value for coronary artery disease *in the individual* (Page[15]).

Decrease of the serum cholesterol in response to feeding fats containing large amounts of unsaturated fatty acids (Ahrens, Blankenhorn and Tsaltas[1], and Kinsell *et al.*[14]) has suggested a role for the latter in the transport of cholesterol (Peiffer and Holman[16]). The unsaturated fatty acids, linoleic, linolenic, and arachidonic, constitute the so-called essential fatty acids. Sinclair has attributed atherosclerosis to a deficiency of essential fatty acids[18].

In view of the great interest concerned with the lowering of serum cholesterol by feeding highly unsaturated fats, it was decided to undertake a study comparing the iodine numbers (degree of unsaturation) of the fatty acids of the various plasma lipid fractions in a group of normal people and those with coronary artery disease proven by the criterion of myocardial infarction. It was reasoned that if a deficiency of unsaturated (essential) fatty acids was a factor in atherosclerosis, a significant difference might be found in the iodine number of at least one fraction. The following plasma lipid fraction iodine numbers were determined: total fatty acids (TFA), phospholipid fatty acids (PFA), cholesterol ester fatty acids (CEFA), triglycerides (TG), and the unesterified fatty acids (UFA).

**Materials and Methods.** The 8 normal ambulatory people used in this study ranged from 22 to 39 years. Two of the 8 were females. The atherosclerotic group comprised 7 patients who were hospitalized and had proven myocardial infarcts of varying duration ranging from 1 week to 5 years. Their ages varied from 57 to 77 and two were females. Blood was drawn from both groups following an overnight fast.

EXTRACTION OF PLASMA LIPIDS. Approximately 50 ml. of blood were drawn from the antecubital vein in heparinized syringes and immediately centrifuged. Fifteen milliliters of plasma were rapidly pipetted into 250 ml. of a 4:1 methylal:methanol solution (Delsal[6]). The flask was held under the hot water tap with cautious swirling until the boiling point was reached, cooled to room temperature, and the material filtered through

*This work was carried out under Research Grants #A–505 and #H–2715 from the National Institutes of Health, Public Health Service.

#1 Whatman filter paper. The precipitate was washed with an additional 50 ml. of the methylal:methanol mixture and the precipitate and filter paper squeezed with a clean glass rod. The solution was then concentrated to 4 to 5 ml. in a 45 degree water bath under a stream of nitrogen. The lipids were separated from the nonlipid substances by vigorous extraction with 30 to 50 ml. portions of boiling petroleum ether (P.E.) at least five times. Each portion was decanted into a 250 ml. centrifuge bottle in a 45 degree water bath and concentrated to 3 to 5 ml. under nitrogen.

ISOLATION OF PHOSPHOLIPIDS. The phospholipids were isolated from the total lipid extract according to Bloor[3]. Fifty milliliters of cold acetone and 20 drops of saturated magnesium chloride were added with vigorous stirring and allowed to remain in the cold for at least ½ hour. The precipitated phospholipids were removed by centrifugation and the acetone supernatant set aside. The precipitate was washed with two 20 ml. portions of cold acetone and the washings added to the original acetone supernatant. The phospholipids were dissolved in moist ether. Two aliquots were removed for quantitative determinations and the remaining solution was saponified.

COLUMN CHROMATOGRAPHIC SEPARATION OF THE ACETONE-SOLUBLE MATERIAL. The acetone supernatant consisting of cholesterol esters, free sterols, triglycerides and unesterified fatty acids was evaporated in a 45 degree water bath under a stream of nitrogen and the residue dissolved in 3 to 5 ml. of warm 1% ether in P.E. This was applied to a silicic acid column which had been washed with 70 ml. each of methanol, acetone, ether, and P.E. (Fillerup and Meade[9,10]). The sides of the column were washed down with an additional 5 to 10 ml. of 1% ether in P.E.

Cholesterol esters were eluted first with 250 ml. of 1% ether in P.E. Preliminary studies showed that 250 ml. of the solvent solution were sufficient for separation of cholesterol esters as further elution resulted in a negative Lieberman-Burchard reaction indicating the absence of sterol.

Triglycerides were next eluted with 300 ml. of 3% ether in P.E. and the unesterified fatty acids were finally eluted with 225 ml. of 50% ether in P.E. Unlike the report of chromatographic separation of lipids (Fillerup and Meade[9,10]), we found that 50% ether in P.E. eluted the free sterol along with the unesterified fatty acids. The latter were separated from the free sterols by the addition of dilute NaOH to form the sodium salts of the acids; the cholesterol removed with P.E., and the fatty acids liberated by acidification.

The chromatographic apparatus was equipped with nitrogen as positive pressure and the collection flask was supplied with a nitrogen inlet.

SAPONIFICATION. Cholesterol esters were saponified by refluxing with 0.7 ml. saturated KOH and 20 ml. absolute ethanol under nitrogen for 60 minutes on a steam bath. The flask was then cooled in ice and 5 ml. each of water and ethanol were added. The liberated cholesterol was removed with P.E. using at least six 20 ml. portions. A qualitative Lieberman-Burchard test was done on the last extraction to make certain all the cholesterol had been removed. Unless an unusually high cholesterol content was present, six washings proved sufficient. The fatty acids were liberated with 5 ml. 10N sulfuric acid and were extracted with P.E. Quantitative and iodine number determinations were done in duplicate directly on the unsaponified triglycerides.

Three milliliters of plasma were used for duplicate determinations of total fatty acids and their iodine numbers. Free and total cholesterol determinations were done in duplicate according to Schoenheimer-Sperry[17] on 1 ml. of plasma.

All quantitative fatty acid determinations were done by the method of Bloor[4] and the iodine numbers according to Yasuda[19]. The isolated cholesterol from the saponified cholesterol esters and the free cholesterol eluted from the silicic acid column were determined by the Bloor method[4].

**Results.** Table 1 shows the iodine numbers of the isolated fatty acid fractions of normal plasma. When the mean values were compared for each fraction, the greatest degree of unsaturation was found in the cholesterol esters. The triglyceride average was slightly higher than the phospholipid, although for practical purposes both can be considered similar. This is unlike a previous study which reported the phospholipids to have a higher degree of unsaturation than the triglycerides (Bloor, Blake and Bullen[5]). The smallest degree of unsaturation was found in the unesterified fatty acid fraction.

Table 2 shows the iodine numbers of the same fatty acid fractions of the group with coronary artery disease. It can be readily seen that the iodine numbers do not differ greatly from those of the normal group with the exception of the phospholipid fatty acids where an average iodine number of 123.5 was obtained as compared to 99.7 for the normal group. When submitted to statistical analysis, no significant difference was found. As in the normal group, the unesterified fatty acid fraction had the lowest iodine number.

Table 3 shows the blood level of the total fatty acids, cholesterol and phospholipids of the normal group. In addition, the last column shows the ratio of the cholesterol ester fatty acids to the total fraction. This is a measure of the chain length of the fatty acids of the cholesterol esters. The theoretical values for cholesterol esters containing a $C_{16}$ fatty acid is 40%, a $C_{18}$, 42% and 45% for a $C_{20}$.

TABLE 1.—IODINE NUMBERS OF THE VARIOUS FATTY ACID FRACTIONS OF NORMAL PLASMA

| | | *Fatty Acids* | | | | |
|---|---|---|---|---|---|---|
| *Patient* | *Age* | *Total* | *Chol. Ester* | *Phospholipid* | *Triglyceride* | *Unesterified* |
| RC | 39 | 95 | 136 | 103 | 75 | 61 |
| MS | 33 | 110 | 169 | 119 | 103 | — |
| LC(f) | 29 | 134 | 182 | 94 | 71 | 89 |
| AL | 22 | 103 | 91 | 79 | 112 | 68 |
| LT | 24 | 119 | 109 | 89 | 74 | 104 |
| CC | 24 | 82 | 118 | 133 | 148 | 153 |
| RH | 22 | 94 | 187 | 78 | 158 | 65 |
| FC(f) | 33 | 82 | 166 | 103 | 84 | 53 |
| Mean ± SEM | | 102.3 ± 6 | 144.7 ± 12.7 | 99.7 ± 6.8 | 103.1 ± 17.1 | 84.7 ± 12.6 |

TABLE 2.—IODINE NUMBERS OF THE VARIOUS PLASMA FATTY ACID FRACTIONS OF A GROUP OF PATIENTS WITH PROVEN CORONARY ARTERY DISEASE

| | | *Fatty Acids* | | | | |
|---|---|---|---|---|---|---|
| *Patient* | *Age* | *Total* | *Chol. Ester* | *Phospholipid* | *Triglyceride* | *Unesterified* |
| TB(f) | 71 | 119 | 135 | 105 | 92 | 69 |
| MS | 60 | 110 | 148 | 140 | 70 | 63 |
| AA | 77 | 95 | 139 | 114 | 141 | 49 |
| TC(f) | 70 | 129 | 172 | 128 | 88 | 97 |
| SH | 57 | 91 | 127 | 135 | 72 | 71 |
| LE | 57 | 93 | 155 | 126 | 107 | 48 |
| JL | 71 | 107 | 166 | 117 | 109 | 124 |
| Mean ± SEM | | 105.7 ± 5.4 | 148 ± 6.2 | 123 ± 4.5 | 97 ± 9.3 | 74 ± 10.3 |

Table 4 shows a similar study for the group with coronary artery disease. There was no significant difference in any of the fractions. The ratio of the fatty acids of the cholesterol ester fraction to the total cholesterol ester fraction was 48% as compared to 43% for the normal group. This was due to the high values of 57.5 and 56.7 in patients T.B. and M.S. respectively.

These higher values were due to technical factors and do not represent fatty acids of longer chain length. Too many qualitative tests for cholesterol were performed in these two to insure complete removal of the sterol from the saponified fatty acids of the fraction.

**Discussion.** This work did not demonstrate any significant difference in the iodine numbers of the plasma cholesterol esters, phospholipids, triglycerides, or unesterified fatty acids in a group of patients with proven coronary artery disease as compared to a normal group. This would indicate that the plasma fatty acids in respect to unsaturation were similar in persons with coronary artery disease and normal people.

TABLE 3.—PLASMA LIPIDS OF A GROUP OF NORMAL PEOPLE

| | *mg./100 ml.* | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Cholesterol* | | | *Phospholipid* | | |
| *Patient* | *Total Fatty Acids* | *Total* | *Ester* | *free* | *Total* | *Fatty Acids* | *% Fatty Acids in Chol.Ester** |
| RC | 403 | 260 | 190 | 74 | 253 | 139 | 45.4 |
| MS | 348 | 230 | 132 | 98 | 142 | 98 | 41.6 |
| LC | 278 | 218 | 151 | 67 | 145 | 107 | 44.7 |
| AL | 264 | 136 | 104 | 32 | 66 | 50 | 47.3 |
| LT | 343 | 217 | 159 | 58 | 116 | 107 | 46.7 |
| CC | 388 | 176 | 107 | 69 | — | 28 | 40.3 |
| RH | 374 | 148 | 94 | 54 | 105 | 65 | 40.3 |
| FC | 501 | 179 | 98 | 81 | 180 | 106 | 43.3 |

*fatty acids of chol. ester / chol. + fatty acids

TABLE 4.—PLASMA LIPIDS OF A GROUP OF PEOPLE WITH PROVEN CORONARY ARTERY DISEASE

| | *mg./100 ml.* | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Cholesterol* | | | *Phospholipid* | | |
| *Patient* | *Total Fatty Acids* | *Total* | *Ester* | *free* | *Total* | *Fatty Acids* | *% Fatty Acids in Chol.Ester** |
| TB | 297 | 208 | 141 | 39 | 93 | 83 | 57.5 |
| MS | 386 | 248 | 170 | 55 | 133 | 90 | 56.7 |
| AA | 374 | 167 | 97 | 32 | 141 | 86 | 47.8 |
| TC | 493 | 309 | 184 | 61 | 172 | 123 | 47.9 |
| SH | 380 | 247 | 156 | 59 | 63 | 36 | 45.2 |
| LE | 602 | 308 | 225 | 68 | 148 | 92 | 43.0 |
| JL | 568 | 261 | 191 | 54 | 171 | 93 | 42.0 |

*fatty acids of chol. ester / chol. + fatty acids

Thus, when the quantitative determination was performed on the combined cholesterol extracts, a smaller percentage of cholesterol was found and so the ratio became higher. Comparison of the chain lengths in both groups showed no difference.

During the past few years, there has been a great deal of interest in the

effect of the unsaturated fatty acids on blood cholesterol levels. It has been well established that the blood cholesterol can be lowered significantly by the ingestion of fats containing large amounts of the unsaturated fatty acids linoleic, linolenic and arachidonic (Ahrens, Blankenhorn and Tsaltas[1] and Kinsell *et al.*[14]). These constitute the so-called essential fatty acids. It has been suggested that atherosclerosis may be caused by a deficiency of essential fatty acids (Sinclair[18]). If this were so, one would expect to find a higher degree of saturation in the iodine number of at least one blood lipid fraction.

It must be pointed out that the iodine number is a composite figure and is a qualitative measure only when a single unsaturated fatty acid is present. It is effected by quantitative factors when two or more fatty acids differing in saturation are present. If a mixture contains a small amount of a highly unsaturated fatty acid, the iodine number will represent an average figure only. The highly unsaturated fatty acid may then be obscured by the larger amounts of fatty acids of greater saturation.

This work agrees with that of James *et al.*[13] who were unable to demonstrate by gas-liquid chromatography any significant difference in the proportions of the various plasma lipid fatty acids in people with proven coronary artery disease as compared to normal people. Hammond and Lundberg[12] found a small decrease in the proportions of dienoic and tetranoic fatty acids (2 and 4 double bonds respectively) of the total polyunsaturated fatty acids of patients with coronary artery disease. However, when this was calculated on the basis of the total fatty acid level of the blood rather than as per cent of the polyunsaturated fatty acids, no significant difference was found. No other studies are available comparing the individual fatty acids or their iodine numbers in coronary artery disease.

James *et al.* used the more precise method of gas-liquid chromatography which measured individual fatty acids[13]. When the above corrections for the work of Hammond and Lundberg are taken into consideration, all three investigations come to the same conclusion. In each study the fatty acids were measured by a different technique: alkali-isomerization, gas-liquid chromatography, and iodine number. A deficiency of essential fatty acids does not appear to be a factor in atherosclerosis.

It must be pointed out that no one has proven conclusively that the fall of plasma cholesterol following administration of unsaturated fats is due to unsaturated fatty acids *per se*. The possibility exists that sterols or other trace materials present may be the responsible factor. Ahrens *et al.* have shown that hydrogenating unsaturated fats diminishes their ability to lower plasma cholesterol[2]. However, any alteration of the fat for experimental purposes may also affect the other factor(s) that could be responsible. Conclusive evidence for the hypocholesterolemic action of unsaturated fatty acids can only be proven by administering the pure fatty acids.

The iodine numbers reported in this study were obtained from the isolated plasma fatty acid fractions, and agree very well with those of Bloor, Blake and Bullen[5]. Although it has been previously reported that the highest degree of unsaturation was found in the cholesterol ester fraction, followed by the phospholipid and then the triglyceride, we found this to be the general rule but not always so. There were one or two individuals in each group in whom the highest degree of unsaturation was found in the phospholipid

or triglyceride fraction. This occurred in two instances in the normal group and once in the arteriosclerotic. In both of the normal people the iodine number of the triglyceride exceeded that of the phospholipid. In the individual R.H. (Table 1), the triglyceride greatly exceeded the phospholipid value but was less than that of the cholesterol ester. Thus, it does not necessarily follow that the highest degree of unsaturation always occurs in the cholesterol ester or phospholipid fraction. In general, the triglyceride iodine number is much less than that of the cholesterol ester or phospholipid fractions.

It should be pointed out that the iodine number of the cholesterol ester fatty acid fraction was determined on the isolated fatty acids after the cholesterol was removed. This obviated a correction factor for cholesterol since it has one double bond and takes up iodine. Bloor determined the iodine number in the presence of cholesterol and applied a correction factor[5].

During the past few years, the blood level of the unesterified fatty acid fraction has been measured (Dole[7], and Gordon and Cherkes[11]). We found this fraction to have the highest degree of saturation in both groups. Dole has reported that oleic, linoleic, palmitic and stearic acids comprise about 80% of this fraction[8]. In one instance, C.C. (Table 1) the unesterified fatty acids had the highest degree of unsaturation of all the fractions.

Although the unesterified fatty acid fraction is small, it appears to be a very sensitive indicator of carbohydrate metabolism, falling when glucose is metabolized (Dole[8]). Apparently a high degree of saturation does not affect the metabolic activity of the unesterified fatty acids to a very great extent.

**Summary.** A study was undertaken comparing the iodine numbers of the cholesterol esters, phospholipid, triglyceride, and unesterified fatty acids of patients with proven coronary artery disease and normal people. No difference was found. This study does not support the concept that a deficiency of unsaturated (essential) fatty acids is of etiologic importance in atherosclerosis.

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## SUMMARIO IN INTERLINGUA

### Le Grado Del Non-Saturation De Fractiones Lipidic Del Plasma In Morbo De Arteria Coronari

Esseva effectuate un studio comparative del numero de iodo pro esteres de cholesterol, phospholipido, triglycerido, e non-esterificate acidos grasse in (a) patientes con demonstrate morbo de arteria coronari e (b) subjectos normal. Esseva trovate nulle differentia. Iste resultato non supporta le conception que un carentia de non-saturate (essential) acidos grasse es etiologicamente significative in atherosclerosis.

## SUMMARIO IN INTERLINGUA

*(See page 336 for original article)*

### Le Effecto Comparative De Agentes Bronchodilatatori Administrate Per Inhalation: Un Studio Clinic A Controlo

Le presente studio esseva interprendite (1) pro determinar le efficacia de 3 drogas bronchodilatatori administrate per inhalation a patientes con chronic asthma o emphysema pulmonar o ambes, (2) pro presentar un meliorate methodo pro le evalutation comparative de plure drogas bronchodilatatori. A fin de assecurar datos de signification statistic ab le studio del plus basse numero possibile de patientes, le 3 drogas e solution salin esseva administrate in 4 differente sequentias con intervallos de 24 horas al 4 patientes in cata un de 4 gruppos que esseva studiate omne die per 1 de 4 differente tests.

Le *activitate bronchodilatatori, i.e.* le Indice BD, esseva mesurate como alteration del grado de compromisso functional post le administration del droga experimental. Le grado de *compromisso functional, i.e.* le Indice CF, esseva registrate coma le procentage medie del predicite valores pro le capacitate vital, le capacitate respiratori maximal, e le capacitate expiratori in 3 secundas.

Le sequente conclusiones pare esser justificate:

1. Le 3 drogas utilisate in le studio ha un melior action bronchodilatatori que le substantia de controlo, solution salin normal.

2. Octo del patientes (*i.e.* 50%) monstrava le melior responsa objective post droga A (Isuprel). Tamen, le datos non revelava un statisticamente significative differentia inter le effectos bronchodilatatori medie producite per le 3 drogas utilisate in le studio (Isuprel, Aerolona, e JB 251).

3. Leve grados de "vertigine," le sol effecto lateral, esseva observate in 2 patientes post le administration de droga A.

4. Dece-tres del patientes (*i.e.* 81%) non poteva recognoscer qual droga produceva in illes le melior resultatos objective.

# ELECTROCARDIOGRAPHIC CHANGES DURING HYPERVENTILATION TETANY

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In a previous study of the electrocardiographic changes caused by hyperventilation only lowering of the R and T waves was found (Scherf and Schlachman[6]). These phenomena were explained by an alteration of the position of the diaphragm which assumes a more inspiratory level after hyperventilation. No parallelism was found between the degree of alkalosis and the changes in the electrocardiogram. Most authors assume, however, that the electrocardiographic changes are due to hypocapnia and alkalosis. According to Lepeschkin, hyperventilation "usually depresses T and RS–T," because of loss of carbon dioxide[5]. Brown, in a discussion of the physiologic effect of hyperventilation, mentions that the characteristic change is a flattening of the T waves and he attributes this to alkalosis[2]. Hyperventilation with alkalosis leads to a lowering of the T waves according to Katz[4], and experiments demonstrating that the changes did not appear when the patients hyperventilated in a carbon dioxide rich atmosphere seemed to prove that alkalosis actually is responsible (Barker, Schrader and Ronzoni[1], Christensen[3]).

Because of these contradictory statements we decided to reinvestigate the problem and to study it in individuals without evidence of cardiovascular disease who were hospitalized for a variety of illnesses. The electrocardiogram was recorded in the standard leads and $V_2$ and $V_5$ after hyperventilation.

**Method.** Hyperventilation was performed by each individual in two ways. A normal electrocardiogram was obtained in each individual after a rest period of 20 minutes. At first hyperventilation was performed by slow and deep breathing, particular care being taken that the expiration was prolonged. This was continued until tingling of the fingers, a positive Chvostek sign or tetany appeared. This was obtained after 3 to 7 minutes. The electrocardiogram was registered and afterward the hyperventilation discontinued. After the patient had breathed normally for 20 minutes another electrocardiogram was registered. The patient was then requested to hyperventilate again but this time with rapid shallow breathing until the same sensations and signs appeared. The electrocardiogram was then repeated. Great care was taken to standardize the tracings repeatedly, to have the patient rest comfortably always in the same position, and to prevent intake of food and drinks between the single experiments.

**Results.** The results are compiled in Table 1. Twenty individuals were tested. With the exception of an occasional slight lowering of the T waves no changes were seen. There were no depressions of the RS–T segment. A study of the literature reveals that the

TABLE 1

| *No.* | *Initials* | *Age Sex* | *Diagnosis* | *Maximum Changes After Slow Hyperventilation* | *Maximum Changes After Rapid Hyperventilation* | *Signs of Tetany* |
|---|---|---|---|---|---|---|
| 1. | A.S. | 34M | After upper respiratory infection | Rate: 75–98<br>T1 down 1 mm.<br>T down in V2 1 mm.<br>T down in V5 1 mm. | Rate: 75–97<br>T down in V2 1 mm. | Chvostek<br>Tetany<br>Tingling of fingers |
| 2. | E.A. | 25F | After upper respiratory infection | Rate: 88–93<br>T up in V2 1 mm.<br>T up in V5 ½ mm. | Rate: 88–96<br>T up V2 1 mm. | Chvostek<br>Tetany<br>Tingling of fingers |
| 3. | G.J. | 22M | Dermatophytosis | Rate: 73–100<br>T1 down 1 mm.<br>T2 down ½ mm.<br>T down in V5 ½ mm. | Rate: 73–136<br>T1 down ½ mm.<br>T down in V2 2 mm.<br>T down in V5 ½ mm. | Chvostek<br>Tetany<br>Severe muscular tremor |
| 4. | S.M. | 33F | Had poliomyelitis months ago | Rate: 93–100<br>No changes of T waves | Rate: 93–125<br>No changes of T waves | Chvostek<br>Tetany<br>Severe muscular tremor |
| 5. | B.L. | 20F | Dysmenorrhea | Rate: 96–125<br>T1 down 1 mm.<br>T2 down ½ mm. | Rate: 96–120<br>T1 down ½ mm.<br>T down in V2 1 mm.<br>T down in V5 1 mm. | Chvostek<br>Tetany<br>Tingling of fingers |
| 6. | G.J. | 21M | Controlled diabetic | Rate: 88–98<br>T1 down 1½ mm.<br>T up in V2 1½ mm. | Rate: 88–100<br>T1 down 1 mm.<br>T up in V2 2 mm. | Chvostek<br>Tetany<br>Tingling of fingers |
| 7. | S.G. | 19F | After upper respiratory infection | Rate: 68–75<br>No changes of T waves | Rate: 68–75<br>T down in V2 1 mm. | Chvostek<br>Mild tetany<br>Tingling of fingers |
| 8. | O.S. | 14M | After upper respiratory infection | Rate: 100–104<br>T up in V2 1 mm. | Rate: 100–128<br>T up in V2 1 mm. | Chvostek |
| 9. | P.L. | 30F | Peptic ulcer | Rate: 76–96<br>T1 down 1 mm. | Not done | Chvostek<br>Tetany<br>Tremor |
| 10. | L.V. | 23M | Slipped disc | Rate: 68–72<br>T1 down ½ mm. | Rate: 68–70<br>T up in V5 1 mm. | Chvostek<br>Tingling of fingers |
| 11. | S.V. | 20M | Homologous serum jaundice | Rate: 68–70<br>No changes of T waves | Rate: 68–70<br>No changes of T waves | Tingling of fingers |
| 12. | A.F. | 22M | Post pneumonia | Rate: 75–88<br>T1 down 1 mm.<br>T down in V2 1 mm. | Rate: 75–88<br>T down in V2 2 mm. | Chvostek |
| 13. | P.C. | 18M | Post pneumonia | Rate: 60–89<br>T1 down 1 mm.<br>T2 down ½ mm. | Rate: 60–90<br>T1 down 1 mm. | Chvostek |

TABLE 1—(*Continued*)

| *No.* | *Initials* | *Age Sex* | *Diagnosis* | *Maximum Changes After Slow Hyperventilation* | *Maximum Changes After Rapid Hyperventilation* | *Signs of Tetany* |
|---|---|---|---|---|---|---|
| 14. | R.J. | 29M | Upper respiratory infection | Rate: 56–70<br>T1 down 1 mm.<br>T up in V2 1 mm. | Rate: 56–75<br>T1 down 1 mm.<br>T2 down 1 mm.<br>T down in V2 1 mm. | Chvostek<br>Tetany |
| 15. | M.O. | 21F | Upper respiratory infection | Rate: 97–97<br>T up in V2 1 mm. | Rate: 97–97<br>No changes of T waves | Tingling of fingers |
| 16. | J.P. | 33M | Post pneumonia | Rate: 88–88<br>No changes of T waves | Rate: 88–88<br>No changes of T waves | Chvostek |
| 17. | V.M. | 17M | Post pneumonia | Rate: 100–100<br>T1 up ½ mm.<br>T2 up 1 mm.<br>T3 up in V2 1 mm.<br>T up in V5 2 mm. | Rate: 100–120<br>T up in V2 ½ mm.<br>T up in V5 1 mm. | Chvostek |
| 18. | B.N. | 33M | Upper respiratory infection | Rate: 83–97<br>T1 down ½ mm.<br>T2 down 1 mm.<br>T3 down ½ mm.<br>T down in V2 1 mm.<br>T down in V5 1 mm. | Rate: 83–100<br>T down in V2 ½ mm.<br>T down in V5 1 mm. | Chvostek |
| 19. | H.M. | 20M | Infectious hepatitis | Rate: 55–79<br>T1 down ½ mm.<br>T up in V2 1 mm. | Rate: 55–82<br>T1 down 1 mm.<br>T up in V2 1 mm. | Chvostek |
| 20. | E.B. | 23F | Post pneumonia | Rate: 97–126<br>T down in V2 1 mm. | Rate: 97–126<br>T down in V2 1½ mm. | Chvostek |

latter phenomenon was only observed in those individuals who developed a marked increase of rate after hyperventilation and it is probable that the tachycardia caused the depression. In some experiments the depression was lacking in spite of an appreciable increase of rate. In view of the regular appearance of signs of tetany in all patients, the scarcity of the electrocardiographic changes is remarkable. It should be stressed that the age of the patients examined varied between 14 and 34. It is possible that with patients in an older age group an increase of rate after hyperventilation would lead to more marked electrocardiographic changes.

**Discussion.** From these results it may be concluded that tetany may appear during hyperventilation without such manifestations in the electrocardiogram as inversion of the T waves and depression of the RS–T segments. It is, therefore, not justified to state on the basis of those experiments in which such alterations did appear that "alkalosis and tetany cause" the above mentioned electrocardiographic changes. They do it only occasionally. The results obtained by reliable investigators cannot be denied. It seems to us, however, that they do not permit generalizations which are so common in electrocardiography. It is certain, in some individuals, that tachycardia leads to

a marked lowering or even inversion of the T waves. It is also certain that, after a heavy meal, some individuals show similar changes. Others have inversion of the T waves in standard leads or some chest leads in the erect posture or after smoking even only one cigarette. Often an increase of rate alone will be responsible in these conditions but sometimes other factors must also be considered. The mechanism by which an increase of rate causes these changes in some individuals but not in others is unknown. It will be important to learn whether the anatomical position of the heart is the responsible factor.

**Conclusions.** In 20 individuals, 14 to 34 years old, the electrocardiogram was studied after hyperventilation. Only minor alterations of the T waves were seen in spite of the regular appearance of signs and symptoms of tetany. The electrocardiographic changes were the same when hyperventilation was done with rapid or with slow breathing.

It is, therefore, improper to state that hyperventilation and alkalosis "cause lowering of the T waves and depression of the RS–T segment." These changes appear after hyperventilation in some individuals but for unknown reasons they are absent in many others.

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## SUMMARIO IN INTERLINGUA

### Alterationes Electrocardiographic In Tetania Per Hyperventilation

In 20 individuos, de etates de inter 14 e 34 annos, le electrocardiogramma esseva studiate post hyperventilation. Solmente minor alterationes del undas T esseva vidite in despecto del apparition regular de signos e symptomas de tetania. Le alterationes electrocardiographic esseva le mesmes sin reguardo a si le hyperventilation esseva effectuate per respiration rapide o per respiration lente.

Per consequente il non es justificate asserer que hyperventilation e alcalosis "causa un abassamento del undas T e un depression del segmento RS–T." Tal alterationes occurre post hyperventilation in certe individuos, sed, pro rationes que es non cognoscite, illos remane absente in multe alteres.

# PROGRESS
# OF
# MEDICAL SCIENCE

## PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

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## THE CONCEPT OF FIBRINOID*

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THE terms "fibrinoid degeneration" and "fibrinoid substance" or "desmoider Faserstoff" were introduced in 1880 by Neumann[51]. He defined fibrinoid as a chemical change of the intercellular substance of connective tissue, causing swelling, homogenization and conversion into a fibrin-like substance ("eine mit Aufquellung und Homogenisierung verbundene chemische Veränderung der Intercellularsubstanz des Bindegewebes . . . welche dieselbe einer Faserstoffmasse ähnlich macht"). Although fibrinoid is found in a number of clinical conditions it is a feature of the diffuse connective tissue or collagen diseases. However, lesions found in collagen diseases, for example, Aschoff bodies and rheumatoid nodules, may also contain hyalinized collagen or necrotic tissue that has been misinterpreted as fibrinoid substance. This has been a source of confusion in recent years. Most authorities now agree that the term fibrinoid should be reserved for an intensely acidophilic (eosinophilic), homogeneous, dense and refractile substance with staining characteristics similar to those of fibrin.

In the long interval since Neumann's publication much investigation concerning the pathogenesis of fibrinoid has failed to resolve the conflicting opinions regarding the origin and nature of fibrinoid. The principal theories postulate its formation from either collagen, ground or cementing substance, smooth muscle, nucleoprotein or fibrin.

*This work was done while the author was at Queen's University, Kingston, Ontario.

Neumann believed that fibrinoid developed from a degeneration of collagen fibers which then acquired the staining characteristics of fibrin. This hypothetical process of "fibrinoid degeneration" in pre-existing tissues, and the substance "fibrinoid" were shown by Neumann to occur mainly in the loose connective tissue of mucous and serous membranes, and in the cardiovascular system. Some of these structures described by Neumann, however, for example, diphtheric pseudomembranes, material in perforated aortic aneurysms and verrucous endocarditis, were at a later date clearly recognized to be deposits of fibrin.

A few years later Marchand[36] brought forward rather convincing evidence that the deposition of fibrinoid occurs during or at a certain stage of fibrinous inflammation. Despite this, the earlier term and concept of fibrinoid *degeneration* persisted. This was not so much because of Neumann's further publications[52,53], or Ricker's[55] demonstration of a fibrinoid degeneration in hygromata, degenerative fibromata and dermoids, but mainly because of the role ascribed to fibrinoid in the evolution of rheumatic diseases by Klinge (1929–33)[29-35]. From his extensive studies on the nature of rheumatic fever, Klinge came to believe that fibrinoid was formed as a result of degenerative changes in the intercellular ground substance. Klinge thought that this process begins as a swelling ("Verquellung") of the interfibrillar ground substance, and is associated with the development of an affinity of the collagen fibrils for silver (argyrophilia), and an increased acidophilia. In Klinge's view fibrinoid differed from fibrin, although they are stained similarly with the common chromatic stains. Occasionally, Klinge admits, exuded fibrin appears to contribute to the development of fibrinoid. He believed he had differentiated between fibrinous exudate on the surface of the synovialis and fibrinoid degeneration in the substance of the synovialis. However, in his photomicrographs, this distinction is not discernible; the fibrin on the surface and the fibrinoid degeneration in the depth appear to be continuous, and Klinge admits that the border between the two is often difficult to define.

Bahrmann (1937)[5] was in full agreement with Neumann that "fibrinoid degeneration of collagen" occurs following injury. He concluded that fibrinoid was caused by a "Zusammensinterung" (collapse) of the collagen fibrils within the collagen fiber. Bahrmann admitted at the same time that swelling and separation of collagen fibers can result from an impregnation of fibrin, causing a picture resembling Weigert's coagulation necrosis. He also believed that transition forms between the fibrinoid of "collagen collapse" and that of "collagen swelling" occurred frequently. Wu[63] reported from the same institute that following a simple mechanical injury (incision) of the skin, the collagen fibers undergo fibrinoid degeneration.

Klemperer, Pollack and Baehr (1941–1942)[22,23], and Klemperer (1948, 1950)[24,25] stressed the fact that the tissue alteration common to the so-called collagen diseases was fibrinoid degeneration. At the same time, Klemperer emphasized that fibrinoid is not specific for hypersensitivity reactions, and may occur in a number of heterogeneous conditions, including, as had been shown previously, in the base of peptic ulcers[1], placentae[19], and the arteries of experimental hypertensive and renal disease. Klemperer *et al.*[22] described fibrinoid as "a deeply eosinophilic substance disposed among the fibers. This substance stains red with azan, and deep mahogany brown with

the Bielschowsky's silver stain. On casual inspection, one might dismiss this alteration as the precipitation of a protein-rich edema fluid. It seems to differ from mere edema, however, in that it is not vacuolated and appears much more substantial than even the most dense of fluid precipitates. We, therefore, are inclined to believe that we are dealing with a physico-chemical alteration of the interfibrillary ground substance. This interpretation is supported by the fact that the interfibrillary alteration is associated with a striking and unequivocal change within the fibers themselves. These lose their delicate wavy appearance; they become straight and irregularly thickened. They are intensely eosinophilic and highly refractile. Their massiveness is augmented by the accretion of adherent clumps of ground substance."

Von Albertini (1943)[1] believed in a dual origin of fibrinoid: that it could arise as a result of degeneration in connective tissue or through alteration of a fibrinous exudate. "Fibrinoid degeneration embraces two distinct phenomena: firstly, the impregnation of the connective tissue with exuded fibrin and the transformation of the latter into fibrinoid; secondly, a primary fibrinoid degeneration of connective tissue fibrils." He subsequently (1953)[2] extended his views with the statement that he could readily separate fibrinous exudates or insudates (Rössle) from "fibrinoid degeneration" and that the former did not belong to what has been termed "fibrinoid degeneration of connective tissue."

The early view of Marchand that fibrinoid is formed from fibrin was revived by Meyer in 1950[42] when he extended his previous observations[39-41] in glomeruonephritis, thromboaniitis obliterans, and arteriosclerosis, to those on fibrinoid in general. He concluded that fibrinoid is exuded fibrin, and is the result of an interstitial fibrinous inflammation. As a pupil of Rössle, he attempted to integrate fibrinous inflammation with serous inflammation under the term "Formenkreis dysorischer Vorgänge." Brockhaus[9] stated that a similar process is responsible for the formation of fibrinoid in vessels in epidemic enteritis necroticans. Böhmig and Klein[7] produced evidence for the fibrinous nature of fibrinoid in valves in rheumatic fever and bacterial endocarditis. The term "fibrinous insudation" was used by the last four investigators.

Singer and Wislocki (1948)[56] showed that under controlled staining conditions the affinity of placental fibrin and fibrinoid for acid and basic dyes was identical. They concluded that placental fibrinoid derives from fibrin. Bussany-Caspari[13] stated that placental fibrinoid is inspissated and aged fibrin. Both fibrin and fibrinoid were digestible with trypsin.

A new approach in the investigation of the nature of fibrinoid was inaugurated with the studies of Altshuler and Angevine (1949)[3]. These investigators attempted to elucidate the problem with the aid of histochemical methods. Their material included samples from the collagen diseases, gastric ulcers, placentae and arteriosclerotic lesions. They extended earlier studies of Klemperer[24], by attempting to show that fibrinoid and metachromasia occur in close topographic relationship in all the enumerated conditions. They postulated that fibrinoid is neither altered collagen nor exuded fibrin, but precipitated acid mucopolysaccharide of the connective tissue ground substance. Altshuler and Angevine believed "the precipitant in some instances is probably an alkaline protein derived from the necrosis of tissue or the interaction of the tissue with the damaging agent." The alkaline protein gave a positive

arginine reaction. Carbohydrate was demonstrated in fibrinoid with the aid of the PAS reaction. "The reaction with phosphotungstic acid hematoxylin has ruled out fibrin as an invariable component."

Following the lead of Altshuler and Angevine, other histochemical and histoenzymatic studies have been reported. Glynn and Loewi[18] studied the histochemistry of fibrinoid in subcutaneous nodules of rheumatic fever and showed that it differs from fibrin in possessing a fibrillar structure and by its resistance to digestion with trypsin. It differed from collagen in its intense PAS reaction and its argyrophilia, but resembled collagen in its digestion by collagenase, and by trypsin after treatment with heat or urea. Glynn and Loewi concluded that "fibrinoid change is brought about by the infiltration of collagen with a polysaccharide-rich material, possibly a glycoprotein." Fawns and Landells[15] studied the fibrinoid in rheumatoid nodules, mainly by histoenzymatic methods. Collagen was shown to be removed by treatment with collagenase and the "granular fibrinoid material" by trypsin. Fawns and Landells were of the opinion that the quantity in which fibrinoid is found makes it improbable that fibrinoid results from a pathological change in pre-existing, that is, connective tissue material. They believed, rather, that the new material which had been incorporated into the nodule must be adventitious. They did not believe that it was fibrin. The novel suggestion was made that fibrinoid, compared to the pre-existing material, is new in components (polysaccharide, tyrosine) and increased in quantity, and is, therefore, more likely to represent "a synthesis resulting from cellular activity than a passive degradation or accumulation of material." Fawns and Landells believed the fibrinoid was produced by the corona cells. However, they could not explain adequately the fact that fibrinoid may be found away from corona cells. Wagner[60] carried out histoenzymatic studies on fibrinoid from various sources. Fibrin fixed by various methods could not be lysed with fibrinolysin (plasmin) but was digested by trypsin. Similarly, fibrinoid fixed in Carnoy's fixative or by freeze-drying and acetone was not acted upon by fibrinolysin but was susceptible to tryptic digestion.

A further possible extrinsic source of fibrinoid has been suggested recently by Klemperer and co-workers[17,26,27,28]. Using histochemical techniques, they found that the hematoxylin bodies of lupus erythematosus contain partly depolymerized deoxyribose nucleic acid. Tissue studies by Klemperer, and Gueft and Laufer had led them to the conclusion that the fibrinoid in disseminated lupus erythematosus has its origin in the protein fraction of nucleoproteins, following depolymerization and release of deoxyribose nucleic acid. "The histochemical analysis," say Gueft and Laufer[17], "suggests that the fibrinoid glomerular alteration in systemic lupus erythematosus might be determined by deposition of degraded nucleoprotein." Gueft and Laufer arrived at this conclusion primarily because they observed the association of the smudgy type of degraded nuclear material, often in conglomerates with eosinophilic masses. The hematoxylin bodies were seen in the lumina of blood vessels, which would indicate that degraded nucleoprotein circulates in the blood, from where it may be deposited into various tissues.

Chemical, biophysical and electron microscopic methods have also been applied to the study of fibrinoid. Kellgren and co-workers (1951)[21], by simultaneous use of histochemical stains, Roentgen-ray diffraction dia-

grams and electron microscopy on the same section of rheumatoid nodules, have shown that the Roentgen-ray diagram and the ultramicroscopic structure of fibrinoid differ greatly from that of collagen which exists side by side with it in the same section. Also the Roentgen-ray diagram differs from that of fibrin. Wolpers[62], on the other hand, in an electron microscopic examination of collagen from the necrotic portions of rheumatoid nodules showed that the cross-striations of fibrils are preserved but that they are slightly thickened and adhere to each other. This phenomenon, he attributed to the deposition of fibrin between the fibrils. Jobst[29] treated a number of lesions containing fibrinoid with phenol or pyridine and examined them in polarized light. Phenol or pyridine causes a change in collagen from positive to negative double refractiveness. On the other hand, the double refractiveness of fibrin is not affected by these substances. Fibrinoid in placentae behaved like pure fibrin, fibrinoid in bursae, ganglia and vessels, like a mixture of fibrin and collagen, whereas fibrinoid in lesions of rheumatic fever seemed to be composed of collagen only. It was concluded that in the latter instance the change characterized by eosinophilia and swelling is due to a change in the link between micellae and the ground or cementing substance of collagen. Consden, Glynn and Stanier[14] investigated the chemical composition of subcutaneous nodules from rheumatic patients. In addition to collagen and elastin, the nodules contained a tyrosine-rich substance and polysaccharide. These substances were present also in normal connective tissue but in much smaller concentration. The presence of fibrin was regarded as improbable. These studies led Consden *et al.* to conclude that there is little reason to believe that such nodules contain substances not present in normal tissue. Ziff *et al.*[64] analyzed fibrinoid, separated from rheumatoid nodules by treatment with either dilute alkali or tryptic digestion, for its hydroxyproline content. They found only traces of hydroxyproline comparable to the amounts found in extracts of normal skin. Ziff *et al.* concluded that the composition of fibrinoid differs from that of collagen and does not include collagen protein as such.

In recent years several studies have been published dealing with the origin of fibrinoid. The studies of Brunson *et al.* (1955)[10,11] deal with fibrinoid in the generalized Shwartzman reaction. These investigators concluded that fibrinoid in the Shwartzman reaction is derived from plasma proteins. They believed this exuded protein represented abnormal proteins. Coincidental alterations of the endothelium of the cardiovascular system were also believed to be of significance. Similar lesions to those of the generalized Shwartzman reaction were encountered in "systemic fibrinoid disease" in man[12]. Thomas and coworkers[57,58] showed that an abrupt and marked depletion of fibrinogen occurred in the circulating blood when fibrinoid was deposited in the tissues. At times there was complete disappearance of fibrinogen. The Shwartzman reaction could be elicited with liquid or other acidic polymers, given instead of the second dose of endotoxin. It was suggested, therefore, that an unknown acid polymer, possibly originating from polymorphs, may play the same role as liquid in the classical Shwartzman reaction, caused by two successive doses of endotoxin derived from Gram-negative bacteria. However, they could not demonstrate the sudden depletion of fibrinogen in the classical reaction. It

was postulated that fibrinoid deposition occurred more gradually, whereas with the combination of endotoxin and liquid the glomerular capillaries become stuffed with fibrinoid within a few hours. Booth, Muirhead and Montgomery (1956)[8], studying lesions of the Shwartzman reaction, attempted to prove that fibrinoid material in the vascular lesions of the Shwartzman phenomenon is derived from necrotic smooth muscle. They showed that a histochemical similarity exists between the composition of smooth muscle and the fibrinoid in the Shwartzman reaction. However, in their hands, fibrinoid gave a much more intense reaction with special staining methods and histochemical methods. They concluded that "the histochemical tests are not quantitative and the apparent increment in material attendant on the 'fibrinoid' change may be due to an unmasking associated with the lesions." In a subsequent publication the same investigators[49,50] injected autolysed smooth muscle (removed from the stomach and colon of dogs) into the renal artery of dogs. This caused embolization of interlobular arteries, arterioles, glomerular and peritubular capillaries. The emboli had the staining and histochemical characteristics of "vascular fibrinoid." The latter, according to Montgomery and Muirhead[45], is different from "connective tissue fibrinoid" (rheumatoid nodules and gastric ulcers). The vascular lesions found in malignant hypertension, experimental hypertension, diabetic kidneys, Shwartzman reaction and thrombotic thrombocytopenia contained a "multiplicity of ingredients, such as fats, fatty acids, phospholipids, aldehyde groups of polysaccharide origin, free potassium, free carbonyl groups, sulfuric ester of mucopolysaccharide origin, and protein bound sulfhydryl groups"[50]. "Connective tissue fibrinoid," as well as fibrin, were found to be relatively free of these substances. However, they were found to contain tyrosine, indole and calcium, which are allegedly absent in the vascular lesion. According to Montgomery and Muirhead, "it appears that vascular fibrinoid and connective tissue fibrinoid from certain sources should be considered fundamentally different."

Gitlin, Craig and Janeway (1957)[16] examined lesions from patients with rheumatoid arthritis, disseminated lupus erythematosus, dermatomyositis, rheumatic fever, periarteritis nodosa, acute and chronic glomerulonephritis, normal placentae and inflamed appendices. An immuno-histochemical method was used, the sections of tissues being studied with fluorescein labeled "rabbit antisera" against human fibrin, albumin and gamma globulin. Fibrin could be localized in all lesions containing fibrinoid. However, they stated "that the conclusion that fibrinoid contains fibrin does not preclude the presence of other substances in the bulk of fibrinoid. . . . The plasma proteins, for example, which are normally present in the interstitial fluid of all tissues, may increase in concentration in areas of inflammation. . . . The large masses of fibrin found and the edema in these lesions strongly suggest that a local increase in vascular permeability is part of the pathologic process, resulting, among other things, in an increase in fibrinogen in the area."

Whereas Gitlin *et al.* did not demonstrate any other plasma proteins in the lesions of the collagen diseases, Mellors *et al.*[37,38], as well as Vazquez and Dixon[59], described the presence of gamma globulin in these lesions. Mellors and co-workers studied particularly the renal lesions of acute disseminated lupus. Gamma globulins were localized in the wire-loop lesions and in hyaline thrombi. These sites

of localization corresponded to sites of "accentuated eosinophilia of glomeruli, as seen in hematoxylin and eosin sections, or with the pattern of PAS-positive areas . . ." They were not referred to as "fibrinoid." Vazquez and Dixon demonstrated the presence of gamma globulin in lesions of rheumatic fever, systemic lupus erythematosus and rheumatoid arthritis. Serum albumin was also shown to be present in these lesions, though to a lesser degree than gamma globulin. Gamma globulin was demonstrated in some lesions, such as the onion-skin lesion of disseminated lupus, which do not contain fibrinoid. The fluorescence shown in one of their subcutaneous nodules is present in the whole central necrotic focus, inside the palisading cells. It has been shown, however, that only part of such a lesion can be referred to as fibrinoid, most of it consisting of necrotic debris[47]. In comparing the distribution of fluorescent areas shown by Mellors *et al.*, and by Vazquez and Dixon with those of Gitlin and co-workers, it becomes evident that the distribution of gamma globulin is much wider than that of fibrin. It is also evident that the latter corresponds more closely to what we call "fibrinoid" in stained sections. Vazquez and Dixon state that their "findings are consistent with the possibility that connective tissue edema, fibrinoid and fibrosis in the above studied diseases have a similar pathogenesis, or that they are different morphologic states in the evolution of a common tissue alteration in which gamma globulin may play a role." They concluded that the localization of gamma globulin in the collagen diseases studied "is a preferential process" and that their "observations are consistent with, but not specific for, the presence of an antigen-antibody reaction in the tissue lesions."

In a review, which included also some original work, Wagner[61] attempted to show that fibrinoid in various conditions derives from various sources. Fibrinoid in rheumatic fever and rheumatoid nodules, Wagner believes, originates from degenerated collagen, in disseminated lupus from degraded nucleoprotein, in the Shwartzman reaction from fibrin, and in malignant hypertension from smooth muscle. Some of this was based on his own observations. He showed that fibrinoid from malignant hypertension was digested by lower concentrations of trypsin than fibrinoid from lupus erythematosus. Fibrinoid from scleroderma required still larger concentration of the enzyme in order to be removed. In connection with rheumatic fever, Wagner refers to his own observations based on the examination of 486 auricular appendages removed surgically during the correction of rheumatic valvular deformities. One hundred appendages were studied histochemically. The fibrinoid of rheumatic fever was found to be "rich in acid mucopolysaccharide material and basic protein which contains alpha-amino acids and sulfhydryl groups. Fibrinoid did not contain succinic acid dehydrogenase, alkaline or acid phosphatase, or TPN diaphorase." The hypothesis was advanced "that with formation of fibrinoid there is dissolution of collagen protein in the ground substance." This was based on both morphological and histochemical observations.

Movat and More[46] examined lesions from various collagen diseases, peptic ulcers, placentae, aortae from arteriosclerosis and also fibrinoid produced experimentally by mechanical (incision), chemical (trypsin), physical (radiation) and allergic injury of connective tissue. A detailed study was carried out on rheumatoid nodules[47].

In addition to morphological and tinctorial, histochemical and histoenzymatic studies were carried out. In both the human and the experimental lesions various stages of fibrinoid formation could be traced from delicate threads of fibrin to bands, clumps and large masses which showed all the morphological and tinctorial properties of fibrinoid. The latter was defined as dense, acidophilic, homogeneous, refractile and showing staining reactions similar to fibrin, that is, red with trichrome or azan, dark purplish-blue with phosphotungstic acid hematoxylin, and red-purple with PAS. With histochemical tests the following amino-acids were identified in fibrinoid: tyrosine, tryptophane, cysteine and cystine. Upon incubation of sections (prepared by freezing-substitution) with collagenase or testicular hyaluronidase there was no digestion of fibrinoid. Collagenase prevented the staining of collagen, and hyaluronidase that of ground substance. Trypsin and fibrinolysin, on the other hand caused complete digestion of fibrinoid and fibrin. It was shown that there are certain amino-acids present in fibrinoid which are also present in blood proteins but are absent in the extracellular component of connective tissue. On account of these, as well as the histoenzymatic and morphological findings, it was concluded "that fibrinoid derives from fibrin which is exuded during the course of a fibrinous or serofibrinous inflammation," and that "there is little evidence that fibrinoid derives from collagen or connective tissue ground substance. Degenerating elements of connective tissue, that is, collagen and ground substance, and also nuclear material and various plasma constituents, may be intimately associated with fibrin in the formation of fibrinoid, but fibrin is the only constant component. Without fibrin there can be no formation of fibrinoid," that is, of the substance referred to as dense, acidophilic, homogeneous and refractile and showing the staining characteristics of fibrin[48].

The most recent publications on fibrinoid deal with fibrinoid in the Shwartzman phenomenon. Miller and Bohle (1957)[43] demonstrated in glomerular lesions of the Shwartzman reaction a periodicity similar to that of fibrin. This periodicity was present also after phosphotungstic acid contrasting[44]. Pappas, Ross and Thomas (1958)[54], on the other hand, showed that the periodicity of this type of fibrinoid was 120A, compared with 230A in fibrin. It was composed of unbranched fibrils. They suggested that fibrinoid in the glomerular lesions of the Shwartzman reaction "may be derived from fibrinogen in a stage of polymerization part way in its conversion to fibrin." The production of fibrils of collagen with periodicities of varying multiples, they stated, may represent an analogous situation.

**Summary and Conclusions.** The literature dealing with the nature and origin of fibrinoid has been reviewed.

In 1950 at the Josiah Macy Foundation Conference on Connective Tissue there was still a considerable amount of controversy and confusion regarding the origin of fibrinoid[6]. In recent years, however, much has been done to clarify this problem. From a survey of the available evidence it appears probable that fibrinoid is not formed by an alteration of extracellular elements of connective tissue but derives from plasma proteins. Of the plasma proteins, fibrinogen is the only one which seems to be present in all lesions, ranging from arteriosclerosis to fibrinoid in placentae, peptic ulcers, generalized Shwartzman phenomenon, malignant hypertension, singers' nodes, so-called ganglia and the various lesions of the systemic connective tissue diseases. These lesions

may be modified from condition to condition and from case to case due to the presence of other substances. While in peptic ulcers, placentae and the glomerular lesions of the Shwartzman reaction, fibrin seems to be the only component, in most of the other lesions additional substances are intermingled with the fibrin. In the systemic diseases of connective tissue and experimental hypersensitivity gamma globulin, probably representing antibody, is deposited along with fibrinogen. Necrotic smooth muscle no doubt is an integral part of fibrinoid in vascular lesions such as malignant hypertension, polyarteritis nodosa and the arterial lesions of the Shwartzman reaction, although fibrin appears to be the main component in these lesions. Although in many lesions, particularly early ones, the connective tissue shows no retrogressive changes, depending on the location and age of the lesion, there may be considerable intermingling of fibrin and other plasma proteins with varying amounts of collagen, reticulum and elastic fibers, ground substance and cellular elements of connective tissue.

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ADDENDUM: After completion of this manuscript a series of three articles on the subject appeared in the French literature. In these Jean de Brux (La Presse Médicale, **66**, 220, 289 and 661, 1958) reaches the conclusion that fibrinoid derives from fibrin.

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## SUMMARIO IN INTERLINGUA

*(See page 269 for original article)*

### Gutta In Femininas Negre

Es reportate le constatationes clinic e pathologic de gutta in un gruppo de 6 feminas negre. Le patientes habeva etates de inter 41 e 71 annos. Illas habeva habite arthritis recurrente durante periodos de inter 3 e 14 annos. In 4 casos le diagnose de gutta esseva confirmate per le demonstration de depositos tophacee in biopsias synovial o in nodulos subcutanee o in ambes. In 2 casos le diagnose pareva esser justificate super le base del observationes clinic in despecto del facto que nulle collectiones de urato esseva detegibile. Nulle del casos exhibiva signos a indicar que le gutta esseva del typo secundari.

Morbo renal de varie grados esseva notate in omne le 6 patientes. Un moriva in uremia. Esseva trovate que illa habeva nephrosclerosis arteriolar e pyelonephritis. Su renes esseva de dimension reducite, e depositos crystallin esseva trovate in le medullas.

Biopsias punceatori del bursa suprapatellar se ha provate de valor per fornir provas pathognomonic in le diagnose de gutta. Es constatate frequentemente que le histos synovial contine depositos revelatori de urato.

In presentar iste casos de gutta, nos desira sublinear le facto que le estimation del incidentia de un morbo particular in varie gruppos racial non significa multo sin informationes relative al segmento del population ab que le datos es derivate e relative al methodos e criterios que esseva usate in le diagnose. In le caso de gutta, il pare que le morbo es minus rar in femininas negre que lo que ha essite supponite in general. Le diagnose de gutta debe esser prendite activemente in consideration in *omne* patientes qui exhibi le symptomas characteristic del morbo.

# PREVENTIVE MEDICINE AND EPIDEMIOLOGY

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## WEATHER AND DEATH

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AN excess mortality regularly accompanies periods of unusually high summer temperatures in the United States. When the thermometer rises above 90° F. on successive days in New York City, deaths sometimes exceed three times the usual number for that time of year. Studies by Collins and Gover in 1934[13] and subsequently by Gover[29] showed this association to hold generally in the United States. Their investigations were based of necessity on grouped weekly data which do not permit full evaluation; the effects of weather on death often are split between parts of consecutive weeks. Other investigations have demonstrated varying degrees of association between summer heat waves and deaths, but commonly they suffer either from restriction of enumerated deaths to those frankly attributable to heat syndromes[3,52,55] or from use of weekly, seasonal or annual data[16,20,30,32].

An early study in Great Britain, that of Guy[32] in 1843, brought out a similar connection between hot weather and high death rates. A hundred years later (1952) these views again had support from Mole[47] who remarks that "a summer heat wave will find the English population unacclimatized and liable, especially after exertion, to the effects of 'tropical' heat."

*Morbidity.* The extent that weather contributes to causation of disease is less definite than its part in mortality. The results of study are divergent despite an interest in weather by physicians since Hippocrates and his Airs, Waters and Places[36]. Morbidity bears on any consideration of weather and death, if only in the sense of more cases, more deaths.

In a study of influenza Herrington and Moriyama[33] concluded that socioeconomic factors were more important than climate in causation. The frequency of all forms of pneumonia in adults was significantly associated with low temperatures and stable weather conditions, when socioeconomic circumstances were held constant. Meteorologic conditions seemed to be a dominant factor in asthma. Markow and Reicher[45], working with monthly weather averages, observed a low activity of symptoms among asthmatic patients in the presence of high humidity, and increased symptoms with a rise in barometric pressure. The results were contrary to those of others who used daily observations of weather.

In 1953, Heyer *et al.*[35] reported that acute myocardial infarct in Dallas, Texas, occurred more frequently in summer than in winter, the reverse of seasonal behavior in northern cities. A difference in the season of greatest physiological stress, as determined by climate, was advanced in explanation[34]. Later analyses led Teng and Heyer[58] to conclude that hospital admissions for coronary occlusion increased with a sudden onset of warm weather, that continued hot weather led to an even greater frequency, and that sudden cold waves produced the highest incidence.

Zink[67] in Hamburg, Germany, noted that such widely divergent conditions as apoplexy, gallstone and kidneystone colic, premature rupture of placental membranes and eclamptic attacks tended to occur with an influx of cold air and dropping temperatures. Tachycardia, preeclampsia, dyspepsia and loss of weight by nurslings increased with unexpected appearance of warm temperatures. Investigations by Undt[59] demonstrated that acute respiratory conditions among subscribers to the Vienna Morbidity Service varied significantly with temperature and humidity, but not with precipitation or other weather variables. Aycock[4] introduced evidence that seasonal and climatic changes influence the susceptibility of the human host to invasion by poliomyelitis virus.

A common belief clearly exists that weather often affects mankind deleteriously, and in manifold ways. Even such matters as the influence of barometric pressure on frequency of motor vehicle accidents have been tested[23]; let it be said, with no evidence to indicate that incidence is any greater on days of pressure change than on days when barometric conditions are stable. Much factual evidence is available in support of an effect by weather on physiological reactions[43,65]. Clinical recognition of such associations is apparent in the common recommendation by physicians of a specific kind of climate for patients with certain diseases[1].

**The Preferred Approach.** The suggestion that weather is one of the factors in the genesis of a number of disease conditions, including acute infections, dates back for centuries. The evidence is rarely sharp, even for the pneumonias which have had more attention than perhaps any other group of diseases. The only American study of deaths in relation to weather, where daily information was available over an appreciable time is that of New York City in the late nineteenth century[40a,41].

Environmental conditions differ markedly now from those then present. The infectious diarrheas of infancy largely have disappeared; they contributed strongly to summer death rates and responded sharply to excessively hot weather. Provisions for general medical care have improved remarkably. Refrigeration and air conditioning modify the physical environment, at least that associated with hot weather

in cities. The relationships, once apparent, may no longer hold.

The preceding summary brings out with reasonable certainty that clinicians and epidemiologists are as much concerned currently with what weather does to established disease, notably the chronic degenerative processes, as with its role in the origin of morbid conditions. Weather in relation to death thus may be approached either through what it does at the start or at the finish of a disease, morbidity versus mortality. The choice here is of weather as a precipitating cause of death.

The decision to take weather as the unit of observation rather than climate, the short term event rather than the long, brings immediate advantage. The appreciable changes and the mixed kinds of meteorological conditions incident to season are avoided. More importantly, the variety of other factors which enter into any complex of causality scarcely comes into consideration. During long periods they change materially. During short periods they are essentially fixed, whatever they are.

An approach through deaths rather than cases avoids the diagnostic errors inherent in identification of persons who are ill; and likewise the difficulty in assuring completeness of numbers. With proof that more people become ill when weather conditions depart abruptly from normal, continued observation is still necessary if the objective is to measure the mortality incident to such circumstances. From a practical standpoint, information about cases is not to be had from existing sources, nor is there adequate control in a knowledge of normal morbidity. The costly procedure of direct field investigation is required to obtain these facts, and the methods are none too good.

The problem is simplified when deaths become the unit of observation. A death is a death, with no equivocation. For the United States and many other countries reporting is essentially complete. The data are readily available and so is a reliable norm over many years.

To set the question anew, the immediate concern is whether or not significant departures from normal seasonal weather patterns produce departures from normal seasonal mortality. For this, no more is needed than total deaths from all causes. Because knowledge is none too definite the emphasis in this review will be as much on what needs to be known as on the information that exists. A plan to acquire the missing facts will be presented.

An affirmative answer to the primary consideration will lead to other and more complicated issues, such as separation of those deaths presumably due directly to weather, identification of disease entities or disease classes at greatest risk, and an assessment of the manner and extent that weather enters into the result.

**Problems Posed.** The acute effects of extreme exposure to cold[22b,61] and to heat[14,35] are well known, those resulting in frostbite[19,22a] or in various heat syndromes[47,66]. The lesser and somewhat prolonged exposures encountered under ordinary living conditions may produce more subtle and less readily identified consequences.

Relationships between the several weather variables and an excess summer mortality are not clear; similar temperatures[20,30] are known to result in material differences in excess mortality. Questions hence arise as to the modifying effect on temperature of other meteorological variables, and the extent of their individual influence in the complex denoted as weather.

The effect on mortality of cold waves in winter has had minor atten-

tion, compared with the summer heat wave. A relatively unexplored question is whether in still other seasons than summer and winter distinctive meteorological variations also affect mortality. The consequence of such events in autumn and spring or as they occur unseasonably at the beginning or end of summer or winter has not been determined, either individually or in comparison with similar episodes at the annual extremes of heat or cold.

**Ecologic Analysis of Death.** As the climax of the disease process, death reflects the interactions[26b] of an affected host, a many-sided environment variously physical, biologic and sociologic, and commonly a recognized specific incitant, the agent of disease. Analysis of the factors that determine the end result differs in no way from methods[28] applied to a determination of origin, or causality; or again those which account for the course that a disease takes. When disease is viewed in its mass proportions, as it affects populations rather than the individual, the problem becomes truly ecologic[26d], and the method of approach is through epidemiology. The present interest is in death as a feature of population pathology[26c].

The determinants of any phase in the natural history of disease rest primarily in two elements, the human host and the surrounding environment. Agents of disease, fundamentally a part of the environment, are arbitrarily separated[26a] to constitute a third element of an ecologic triad, largely because of convenience in fitting ecologic analysis to the pattern of medical diagnosis.

As in most epidemiologic situations host characteristics, collectively recognized as resistance, are the main factors determining when death will occur and under what conditions. Resistance determines whether the balance turns toward a fatal result or toward recovery. The content of resistance ranges from genetically inherited traits to those specifically acquired as antibodies. Others depend upon anatomical nature and the state of physiological functioning. Growth and development contribute strongly.

The present problem resolves into how much and what kind of weather has the potential of overpowering these defenses. The presence of disease of any kind suggests an increased liability to heat effect; the kinds of patients peculiarly susceptible are to be identified. Injuries perhaps serve in the same way, and aberrations in homeostatic mechanisms rather clearly predispose to weather changes, as do age, sex and race.

The environment is a more complex affair than host, if for no other reason than all else besides man is within its scope. The association of man with his fellow man adds a division of environment peculiar to Homo sapiens, a social environment. Death rates show a strong relationship to the social environment, for communities as a whole and for individual diseases. Such social factors as housing, income, education, marital status, occupation and the cultural attributes of minority groups are a part of every study of a population disease, and of deaths. The kind of medical care, its availability and utilization, have had much attention in recent years in a growing appreciation of the significance of social factors.

The biologic environment, both plant and animal, has to do with the dominant features of food supply and nutrition. Beyond that, animals serve at times as reservoirs of infection; in many places they are a determining factor in health and welfare, as beasts of burden; and the ticks, bugs, fleas and flies of this world have no small part in economic welfare, in food supply and in transmission of disease.

Physical environment includes such factors as geographic location, terrain, altitude, and weather; and this is a good place to distinguish weather from climate. Climate is a long-term manifestation, the concerted effect of all these factors. Weather is the short-term combination of meteorologic variables that make today pleasant, hot, gloomy or rainy. The present interest has no concern with the influence of climate on disease or on deaths, except as climate is characterized by differences in weather and weather effect.

Weather is modified in some measure by these other environmental attributes, but as an essentially local phenomenon its origin is outside their general scope. Hence weather as a factor in death can be assessed independently. Generalization of findings to other areas of similar climate is hazardous without considering other features of the physical environment[12,57]; and even more so the generalizations to other populations without account of the totality of factors there active in deaths.

An imposing list of things determines death rates of a community[26a] and emphasizes the principle of multifactorial causation. Diverse and numerous elements ordinarily are to be considered, along with their interrelationships, in judging the significance of any one. That would include weather, were it not for the element of time. The broad matrix of causality changes, but not appreciably within the days that a particular brand of weather is active. With due regard for other unusual and suddenly breaking events conceivably entering concomitantly, as an epidemic of influenza, excess numbers of deaths in a population may be considered for practical purposes as in association with a heat wave or other meteorologic event. The nature of weather is now examined, along with its several variables and the means to measure them.

**Weather Variables.** Weather is a composite of meteorological elements, each with a potential to affect mortality. If the total complex is to be balanced against frequency of deaths, the practical problem is to select among existing variables those judged significant, and yet to keep the number within manageable limits[53]. Since weather is dynamic, constantly shifting in terms of its several components, the need is for values representative of each selected variable which together will describe in adequate fashion the general character of the day's weather. Daily information is necessary, because a particular spell of weather is of varying duration, from a single day to several, or uncommonly for a week or more.

The main elements in weather are temperature, wind direction and wind velocity, hours of sunshine, moisture content of the air, precipitation, and barometric pressure especially as evidenced in cold and warm fronts. Measurements are possible for all and for such lesser known characteristics as cosmic, electrical and magnetic phenomena. Deaths presumably will have closest association with those variables provoking the more profound physiologic stress.

Because some meteorological phenomena modify others, and thus in a sense become dependent variables, their practical measurement is possible by the primary element. For instance, the influence of precipitation may be judged by moisture content of the air, of which precipitation is the upper limit. Wind velocity and wind direction are partially determinative factors of temperature and humidity, and hence reflected in those two elements. Hours of sunshine contribute to the daytime heat and hence affect the maximum temperature. So considered, the

summary effects of weather on death should be measurable in terms of three major weather variables: temperature, barometric pressure and moisture content of the air.

*Temperature.* The United States Weather Bureau through the Department of Commerce publishes temperatures from hundreds of weather stations throughout the country, on an hourly basis for each day of the year[60]. Summary tables give daily maximum, minimum and average values, as well as departures from normal for the prescribed date. The practical question is which measurement best reflects the influence of temperature on death.

Relatively high temperatures with little variation during a 24-hour cycle conceivably have a greater effect on deaths than the same maximum accompanied by a relatively low minimum. Opportunity is less for recovery from the induced physiologic stress. The mean temperature as computed by the Weather Bureau is a simple arithmetical average of maximum and minimum values for the day, measured from midnight to midnight. With equally high temperatures, the day with the wider diurnal variation has the lower average. The converse is true at the low end of the scale.

From weather bureau data daily averages could be calculated from 24 points corresponding to the hourly temperature readings. The added precision appears not worth the lengthy computations. The use of 12 or even 6 points has little advantage over the simple average of high and low readings directly available from weather bureau publications. The ratio of the observed mean on a particular day to the calculated normal for that date provides a workable measure of departure from the expected.

*Barometric Pressure.* The Weather Bureau also publishes barometric pressure readings made each day at specific hours: 7 a.m., 12 noon, 7 p.m. and 12 midnight. No comparable daily long-term averages of barometric pressures are published as for temperature; monthly averages are tabulated. The range is numerically small, from 28.38 to 31.01 inches of mercury during the many years that observations have been made in New York City. Daily, monthly and annual ranges are within still narrower limits. A seasonal pattern is not so definitely discernible as for temperature, although summer pressures are generally higher than those of autumn and winter, and the range is narrowest in mid-year.

The 7 a.m. reading would seem most likely to reflect conditions of atmospheric pressure active during that day, with the monthly average serving satisfactorily as an indicator of the usual. The computed ratio would constitute a measure of departure from the expected for the day, in judgment of an effect on frequency of deaths.

*Moisture Content of the Air.* Two sets of daily measures of moisture in the air also are published by the Weather Bureau; one is relative humidity, the other the dew point. Relative humidity expresses the amount of moisture as a percentage of the amount necessary for saturation, while dew point is the temperature at which moisture would be deposited; in other words, the temperature at which humidity would be 100%. Dew point stands in direct relation to the existing temperature, while relative humidity is independent of that value. For this reason dew point is preferred. As with barometric pressure, the 7 a.m. reading is preferred and the available monthly average judged satisfactory in calculating ratios to depict the departure of the dew point of a specific day from seasonal expectancy. Many combinations of meteorologic elements and the

means for measuring them are possible but the scheme just advanced appears to meet the required needs of an attempt to match weather against deaths.

Attention now turns to the nature and substance of the evidence to justify an hypothesis that weather has an influence on deaths. The information is from two sources, the physiologic reactions of man to various weather stresses, and, secondly, the behavior of deaths in various climates of the world. The response to heat and cold has had the major consideration in physiologic studies.

**Physiologic Responses to Heat and Cold.** Man maintains a constant body temperature by dissipation of metabolic and exogenous heat in excess of energy requirements[49,61]. The four mechanisms of conduction, radiation, convection and evaporation accomplish this purpose. According to Lee[44] thermoregulation involves many, if not all, body systems in a complex fashion, and physiological crisis may occur before a marked change in body temperature is determinable.

*Heat.* At high environmental temperatures, evaporation is the most efficient mechanism for dispersing body heat. Losses by radiation and convection require that the outside temperature be lower than that of the body; at extreme external temperatures this situation does not exist. Moreover, humidity modifies loss by radiation, and wind velocity augments convection[37].

Radiation and convection require carriage of heat to the surface of the body, a process accomplished through vasodilation; cardiac work is thereby increased, as is blood volume, and arterial pressure drops[9,14]. Cook[14] emphasizes that excess weight brings added stress through greater demand on the heart. Capillary-free fat tissue provides more insulation, but the additional surface area is insufficient to dissipate the extra heat. Patients with congestive heart failure commonly react quickly and severely to rapid elevation of temperature and humidity, compared to persons with no evidence of that condition[10].

The importance of sweating is consistently emphasized[18,24a] as a major factor in heat loss during times of high temperature. During acclimatization to heat, sweat is excreted in progressively larger amounts during the first week; and renal retention of sodium and chlorine ions surpasses that required to support these sweat losses[8]. Nevertheless, large salt loss in excreted fluids presumably leads to a decrease in extracellular fluid and plasma volume[35]. Lee[44] asserts that "any failure of the sweating mechanism must result in heat exhaustion or heat stroke," and Friedfeld[24a,b] points out that cessation of sweating is a characteristic premonitory indication of the onset of heat stroke in elderly persons. Among 60 patients treated for heat stroke in the Emergency Room of Beekman-Downtown Hospital[52] in New York City during the September, 1953, heat wave, 9 were admitted to the hospital with symptoms of weakness, nausea, cramps in legs and abdominal muscles, and anuria. Blood examination showed decrease in plasma chlorides and 4 patients had leukocytosis[52].

Patients with fibrocystic disease of the pancreas[14] appear peculiarly susceptible to excess heat because of abnormalities in sweat electrolytes. Rates of sweating among persons with congestive heart failure are decreased by 27 to 47%[44], which may account in part for the severe reaction they experience to heat. Acute manifestations of heat stress occur most frequently among infants and elderly people[14]. Austin and Berry[3] believe that degenerative disease in general contributes

to susceptibility of heat stroke. In view of the stresses incident to maintaining constant temperature, especially those on the cardiovascular system, age differentials in ability to withstand hot weather are to be expected, particularly since other physiologic dysfunction probably will accompany vascular changes in the elderly.

*Cold.* Physiologic mechanisms for maintenance of body heat under conditions of extreme cold have been well studied[44,64], as have reactions to severe cold stress[46,61]. Little information is available on the reaction to more prolonged cold of lesser degree as experienced in such temperate climates as that of the United States.

The means for modification of cold and its effects on man have been more effective or more widely adopted in temperate regions than measures to reduce the consequences of excessive heat. Central heating, adequate clothing with improved insulation, and no usual demand at least in cities for prolonged exposure out-of-doors tend to minimize low temperatures. Since mortality rates are higher in winter than in summer where the effects of excess heat are clearly demonstrated, the relation of winter mortality to variations in weather are worth exploring.

As vasodilation serves to aid dissipation of heat, so vasoconstriction preserves heat within inner body structures. While the outer thermal layer of about 2 cm. manifests a lower temperature in response to cold, that of the inner body may rise[19]. Increased heat production is the only way of preserving deep body temperature[44]. Shivering helps accomplish this, and the basal metabolic rate may rise substantially: in dogs as much as sevenfold[19]. Paradoxically, inner temperature falls with the return of outside temperature to higher levels, through warming of superficial areas and increased blood flow to them. Laboratory experiments have demonstrated that moisture of the air plays little part in the chilling effect of cold[31], in contrast with its action in exposure to heat.

According to Edholm[19], intense chilling primarily affects the outer thermal layer but a marked drop in deep body temperature is reflected strongly on the circulation, on respiratory control and on acid-base balance. Blood pressure falls while blood volume and plasma are reduced. The heart rate may drop to 30 per minute or less, due to cooling of the pacemaker; arrhythmias and atrial fibrillation ensue. Oxygen consumption declines to perhaps one-fifth of normal, but circulating oxygen in the blood may remain essentially adequate for tissue demands. However, no dissociation of oxyhemoglobin occurs and carriage of carbon dioxide is affected. As a result, the acid-base balance changes markedly. Long-continued shivering is fatiguing and distracting[38], fine coordination becomes impossible, and yet it leads to favorable modification of thermo-regulatory vasoconstriction by cardiovascular changes[46]. Other workers[39] question a measurable efficiency of shivering in increasing heat production.

That long exposure to rigorous climatic conditions, over generations rather than months, leads to physiologic changes protective of residents of such areas seems clear. The mean bloodflow in forearm and hand of Eskimos[22b] was found to be higher than for whites, as measured at 68° F. room temperature. The blood pressure of Eskimos increased more in response to cold (immersion) than did that of whites. Such circulatory responses would appear a special adaptation toward withstanding cold temperatures. In the Korean war[22a] during the winter of 1951–52 a greater proportion of

white soldiers from southern United States suffered frostbite than those whose homes were in the north. A similar differential did not hold for southern and northern Negroes. The inference is reasonable that differences between the two white groups rests in knowledge on the part of the northerners of how to take care of themselves, through experience gained in northern winters. That such learning on the part of northern Negroes was overbalanced by inability to adapt from the hot climate enjoyed by their forebears would seem highly conjectural.

**Mortality in Major Climatic Zones.** After long investigation of the influence of climate on health, Ellsworth Huntington[40b] came to the conclusion that the well marked seasonal changes characteristic of temperate climates provide the optimal environment for man. One of his main arguments was that the great major civilizations of the world developed there. Furthermore, changeable conditions in respect to weather[40a], as distinguished from climate, were accompanied generally by a lower mortality than in regions of stable weather.

Crude mortality rates are materially less in temperate climates than in the arctic or the tropics. For example, mortality in the United States[48] was 9.6 per thousand per annum in 1957. In Greenland[17] for 1954 it was 15.3; and for India[17] the rate was an estimated 27.4 per thousand per year in 1950, a figure later substantiated by one of us through actual field survey in one part of India, the Punjab. A lack of reliable data on both deaths and weather of itself precludes a comparison of arctic, tropical and temperate climates. More importantly, the differences in the contributions to mortality from the social and biological environment are so great that such an attempt would be difficult, if not fruitless. The value of studies on short-term departures from established patterns of meteorologic behavior in those areas is another matter.

*The Arctic.* The prevailing low temperatures of Alaska and similar areas of the north are responsible for a variety of indirect effects upon health, but identification of direct relationships between cold and the existing high death rates is difficult. Housing, heating, water supply and waste disposal all suffer by reason of the rigorous climate. Seasonal food shortages occur because of difficulties in communication and transportation, factors also responsible for deficiencies in health service.

The white population in Alaska has no greater mortality than that for the comparable group in continental United States. Deaths from accidents, homicide, suicide and alcoholism are more frequent, presumably due to working and living conditions and to the behavior problems of recent immigrants. Early in this decade infant mortality for the territory was about 52 per 1,000 live births, compared to 29 in continental United States; the rate for white infants was only 24, that for natives was 101. The Parran report[2] on health conditions in Alaska concludes that health and medical facilities appear as adequate for whites as in the rest of the United States, but not for natives. Where the two peoples are intermingled, as in the more populous parts of subarctic Alaska, mortality rates for whites and natives differ no more than those of whites and nonwhites in continental United States. In the more remote parts of the territory, death rates among natives are distinctly greater.

Stefansson[56] reports individual long-lived Eskimos, but some of these lengthy lives are based on hearsay evi-

dence and admittedly tenuous estimates of age. Our own observations in a number of representative parts of the arctic support such occurrences. The facts are nevertheless clear that average life expectancy in most native populations of the arctic is about one-half that of residents of temperate regions. Provided the hazards of infant mortality are successfully avoided, and traumatic injury or an epidemic are not encountered, nothing in the arctic climate or the arctic weather seemingly precludes a good old age.

According to Carles[11], writing of Greenland, the cold, the frequent storms and the variable sunlight of the arctic climate are neutralized to an extent by the extreme dryness of the atmosphere, the bacteriological purity of the air, and the high electrical potential. He likens the tonic and salubrious result to that afforded by high mountains in the temperate zone.

To ascribe the higher mortality in the arctic primarily to cold is unwarranted in view of so many other health hazards. The management of injuries may be complicated by inability to provide external heat in treatment of shock, but this derives as much from the primitive living conditions and the lack of fuel as the circumstance of extreme cold[12]. Similarly, the limited water supply despite an abundance of snow and ice, a circumstance appreciated only by those who know the arctic, presumably contributes to a less favorable outcome of traumatic injury as it does to the frequency of dysentery[6]. These things are not directly a function of climate but of other features of the physical environment[7] and especially the social environment. When other conditions are held constant the adverse effects of the arctic climate are hard to identify.

*The Tropics.* Huntington[40b] points out apparent exceptions to his thesis that optimum temperatures for man lie between 63° and 73° F. Such temperatures prevail in northern India during December, January and February. That mortality is high simply demonstrates the activity of other factors. Some are indirectly related to weather. The heavy breeding of mosquitoes after the rainy season once was an outstanding consideration because of malaria, but mortality still is in excess of 25 per thousand per year in such rural populations as the villages of the Punjab, with malaria well controlled. Huntington sums up the situation in saying that "mean temperature is only one of the climatic factors, climate is only one of the physical factors of environment, and the effects of physical environment are modified by cultural environment."

Waddy[63] presents cerebrospinal meningitis in epidemic form as evidence of a direct relationship between weather variables and a disease condition, specifically lack of rain in the dry season. This acute infection is characterized by extremely high attack rates in equatorial Africa, compared with South Africa or in the United Kingdom. Most epidemics begin about half way through a dry season, and build up slowly thereafter. Only sporadic cases occur during the subsequent rainy period. When the next dry season appears the epidemic commonly resumes at the immediate beginning of that season and tends to be huge, until terminated by exhaustion of susceptibles. Waddy ascribes this pattern to the longer life of airborne infectious agents as well as to known physiologic reactions under conditions of low absolute humidity during the dry season but adds that the construction of houses with little daylight entering contributes to contact spread and dissemination of the infectious agent.

The great malaria epidemic of 193[illegible]–

35 in Ceylon[25] is a good example of the usual succession of events, starting under a climatic influence but in its causative factors extending far beyond. In this instance the beginning was a drought. Small puddles of water collected on dried-up water surfaces, thereby favoring breeding of mosquitoes. Malaria followed. Crop failure was a further result and the subsequent famine led to lowered general resistance of the population, favoring the continued course of the outbreak. The conclusion becomes evident that the origin of few acute diseases, or of epidemics, is directly traceable to weather variations or climatic differences, except as they determine other and commonly more important ecologic variables, a view which Passmore and Sutherland[50] support.

One of the first things evident in the three broad divisions of the world, temperate, arctic and tropical, is the wide differences that exist within them. Desert, jungle and grassland or steppe, require differentiation in any interpretation of mortality and tropical climate[27]. The cold nights of the desert enhance ability to face the heat of the following day. Sweat glands are rested, depleted salt and water restored, and skin and internal temperatures approach normal. In the jungle, the night provides no resting period; the situation actually may be worse than during the day because of lesser movement of air, an important element in the sweating process. These differences require a new acclimatization in transfer from desert to jungle climate, and present a new set of environmental circumstances, although persons acclimatized to the jungle gain in ability to tolerate the desert. Skin diseases[21] commonly plague the unaccustomed under either situation, sometimes prickly heat, or pyogenic or mycotic dermatoses. In addition to the physiologic manifestations earlier discussed, or even in their absence, fatigue is a common symptom of persons introduced to either type of climate. We recall our own skepticism of that complaint when first we entered the tropics, and boldly labeled it an aversion to work. One learns, however. Stefansson[56] has recognized "the friendly arctic;" both extremes of world climate are that, if the effort is to adapt to, instead of fighting them.

Huntington[40b] offers mortality data for Javenese cities at different altitudes in support of the claim that climate and general mortality in a semi-tropical environment are related. Apart from size of community, conditions in the cities were essentially similar insofar as common factors bearing on death rates were concerned. The death rate declined substantially as cities were situated progressively above sea level. Huntington considers more than coincidental that temperatures also decline from a yearly average 79° at sea level to 72° F. at 1800 to 2500 feet altitude, the latter bordering the optimum that Huntington supports.

Meaningful mortality data leading to inferences regarding the effect of climate on deaths are lacking to much the same extent in the tropics as in the arctic. Other environmental factors obscure the possible direct effects of heat and other weather variables. Ceylon again is used in example[15]. With the control of malaria, a decline in mortality occurred within a decade from a level typical of Asian nations to the more favorable rates of Western countries. The morbidity pattern still departs measurably from that standard, with explanation to be sought in differences in diet, in physiologic characteristics, and independent of climate. In the face of these profound changes Cullumbine[15] remarks with much reason that the extent to which mortality,

morbidity, physical and physiologic differences, are related to varying environments: climatic, social, economic, biological, parasitological: is still doubtful.

*Temperate Zone.* Areas in temperate climates can duplicate at times conditions considered only possible in the tropics or the arctic. Temperatures above 120° F. or more than 50° F. below zero are recorded, but with one essential difference. Duration of such extremes is commonly in terms of hours or days, at least in the places where appreciable numbers of people live, rather than seasonal and long continued as in the tropics or arctic. Such temperatures when they occur in the temperate zone expectedly lead to a different result in respect to deaths than those in tropical or arctic environment. There is the important matter of acclimatization.

In most considerations of weather in temperate zones and its impact on death rates, attention has centered on the extremes of winter and summer. In a thoughtful analysis Aycock *et al.*[5] introduced evidence that the gradually changing temperatures in spring and autumn serve a beneficial physiologic purpose through acclimatizing and preparing the body for the annual temperature extremes in prospect, just as night on the desert permits resurgence of ability to withstand the stresses of the day. What needs to be known is the effect of the unusual during those seasons, the spells of hot or cold weather that depart measurably from what is customary, as reflected in deaths of the moment and in the succeeding season of extreme temperatures.

Health, death rates and life expectancy clearly have widely divergent values in different climates of the world. The contribution of climate as such to the observed differences is indefinite. Proper assessment depends upon evaluation of other and wholly unrelated factors entering into the observed result; and, secondly, the extent to which climate exerts an indirect effect[43], through such opposite influences as what people eat and the breeding of mosquitoes. The problem resolves into a proper handling of multiple variables, but that is difficult and expensive. A simpler and more direct approach is suggested through study of weather in its sudden departures from the normal and habitual, a practical matter because all climates and all seasons have their precocious moments.

**Weather as a Precipitating Cause of Death.** In the course of a year, seasonal changes of a regular nature occur in weather factors and in mortality. A chart of mean temperatures superimposed on a chart of deaths shows over a year a simple inverse relationship. It is common knowledge that mortality reaches its peak in the United States in winter when temperatures are low, and its low in summer when temperatures are high. Figure 1 depicts the typical seasonal variations in mortality and temperature for the City of New York. The mortality curve is based on a 5-week moving average of the mean of death rates for the given week over 5 successive years. The temperature line is a smoothed fit to the normal mean for Wednesday of each week as determined by the Weather Bureau. From early March to mid-June, temperature consistently climbs as mortality decreases. From early September until nearly the end of the year, temperatures drop just as regularly while deaths increase, again an inverse relationship but with the trend in the opposite direction. The behavior in spring and autumn is in effect a mirror image, the one of the other

If the absolute changes in tempera

ture over several days in spring are related to absolute changes in daily deaths during the same period the expected finding is that rising temperatures are accompanied by falling mortality. Conversely, in autumn, a rising mortality accompanies falling temperatures.

The general inverse relationship of weather and death is of interest, but absolute values of weather variables are not the likely factors in an existing association. More probably the important consideration is the extent to which a particular weather factor departs from usual expectancy for the prescribed time. In most considerations of weather and its impact on death rates, attention has centered on the extremes of winter and summer. Earlier in this discussion the suggestion was made that the intermediate seasons of spring and autumn have been neglected to the loss of a full appreciation of the problem. Furthermore, the effects of such departures presumably vary, depending upon whether temperatures are generally rising as in spring or falling as in autumn. A separation of data by season is necessary to avoid the possibility of obscuring significant findings. Analysis on an annual basis scarcely suffices.

*Deaths.* Aggregated data on deaths are unsatisfactory as a mortality base against which to measure weather effects, either when determined by weeks as is usual, or worse still when the aggregation is monthly. Daily information by age and sex is needed; the daily requirement because of a frequent split effect between weeks, that by age and sex because of a likely differential. With such information, daily averages for total deaths by age and by sex, may be computed for each month of the year. The ratio of deaths on a particular day or series of days to the monthly average should be a satisfactory indicator of variation from the expected.

The principal objective is to know

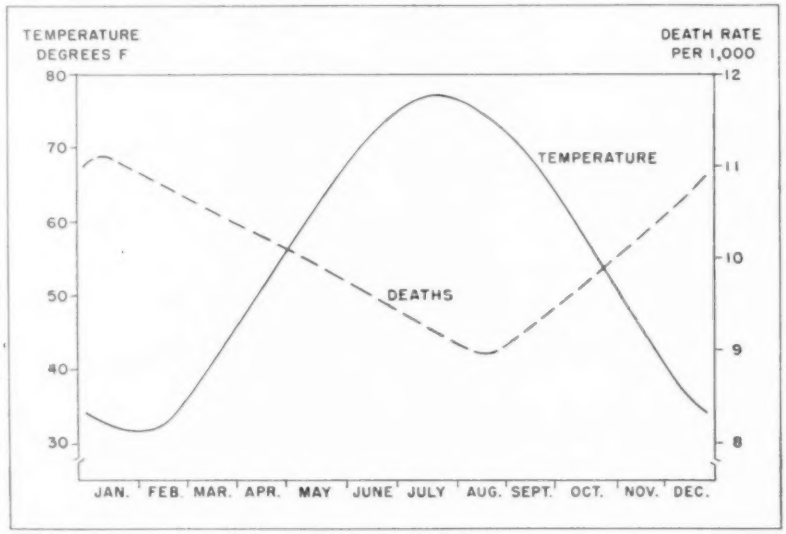


Fig. 1.—Temperatures, and death rates per 1,000 population per year in New York city, mean values 1957.

the effect of short-term unseasonable weather on total deaths. With little added effort and close to equal reliability, data on age and sex are to be had from official records of death. If particular age groups prove unduly vulnerable to the action of weather, then the causes of death peculiar to those ages will be strongly implicated. Further analysis through identification of single causes of death does not appear justifiable without better proof of the ages more liable. Furthermore the recognized fallibility of causes of death as recorded on death certificates, and the frequent failure to include important contributing ailments discounts the value of analysis of this material for association with weather conditions. Fluctuation in specific causes of death according to weather conditions is better approached through field studies than death certificates.

*Weather.* With the hypothesis that unusual, abrupt, unexpected and temporary departures of weather from an established normal lead to unusual numbers of deaths, the practical requirement is now a definition of what is meant by such situations, the characteristics to be evaluated, and the means to measure them[62].

Weather situations such as cyclones, tornadoes or floods[54] are arbitrarily classed as bizarre and ruled out as something more than the unusual. They are catastrophic. The present concern is with the warmer than warm periods of summer, or the cooler than ordinary; and in winter with excessively cold or excessively warm spells; and similarly, of course, with the other variables than temperature or with weather viewed as a composite.

Time of occurrence is an important consideration. A rise in deaths with excess heat of summer, when warm weather is usual, has had much attention. Little information is available about warm spells when cold weather is the rule; for example, the impact on deaths of mean temperatures of 60° F. for several days during winter when temperatures are normally in the low thirties. Such behavior may be beneficial, with lower death rates in accordance with the more favorable rates of summer; or with the body conditioned to low temperatures the effect may be adverse. A continuing consideration is that people in present-day metropolitan communities may well have little need or opportunity to acclimatize to winter cold, what with central heating and warm clothing so generally available. As a consequence an appreciable rise in outdoor temperature might have little or no observed effect; and the aged and ill, presumably at enhanced risk to excessive cold, are especially well sheltered at those times. At any rate, little is known of what results from the transcendent variations that weather takes in winter.

Another practical need is to determine the extent of departure of weather from normal values that ordinarily results in more deaths. A zone of tolerable departure presumably exists within which increased numbers are unlikely. What is needed is the significant level at which that result occurs, further enlarged through sufficient experience to determine the rate of progression in deaths with still higher or lower temperatures. Temperature scarcely can be considered alone because of known modifying effects of air moisture and barometric pressure. If high humidity tends to exaggerate temperature effect, a prescribed temperature may be of small or great consequence dependent upon that alone. The extent to which the several weather variables act in concert and the pattern of their interrelationships is a part of this evaluation.

Some years have no heat waves, or

conversely no cold spells. On other occasions, a succession of such events marks a particular season. The cumulative effect requires definition, including the number of incidents, their duration, and the interval between them. Speculation can lead to the view that susceptibles are eliminated in the first wave with fewer deaths in the next episode; or that tolerance is engendered, with the same result. The opposite reasoning predicates an overwhelming of resistance with succeeding episodes, with increasing numbers of deaths. The facts are just not known.

The reviving stimulus of cooler weather interspersed with extremes of heat has been noted earlier. From this it could be reasoned that a single day of exceptionally high mean temperatures might be of little consequence, and that a succession of such days is required for a noticeable result in excess deaths. This brings the logical query of how many days. An allied consideration is the relative aftermath of long-continued low excess of seasonable weather compared with a high excess of short duration.

Rate of development of a weather anomaly is a characteristic independent of actual extent. Sudden rises of 20 or 30 degrees in temperature, even though the higher level is not excessive, may have significance because of the need for rapid adaptation. This situation presumably would be exaggerated with a combination of both abrupt rise and high level.

Related to the question of how long a heat wave or other weather event must last to produce a significant change in mortality is that of where to look for the consequences; on the specific days of occurrence, either more or less at the start or the end, or a delayed result extending perhaps over the next several days or even a week. The need is for an intensive review of enough episodes to establish the principle of an immediate, a progressing or a lag effect.

**Plan for Action.** Most of the considerations just presented were a part of the outline that initiated this review of weather and deaths. One or two were added as the argument took shape. Realization came rather promptly that evidence on most of these matters did not exist. Decision followed to undertake an investigation in New York City of experience of the past 10 years based on the considerations just advanced. The practical benefits justify the effort: the usefulness of the information in advice to the public and to hospitals by health authorities and the kinds of patients and persons to protect in such junctures.

The important requirements for such an investigation appear to be a defined and limited area of such size as to have more or less uniform weather throughout its extent; where the consequences of variables other than weather can be construed as relatively constant; a population of sufficient magnitude to permit evaluation in significant terms within a reasonable period, for the broad character of weather also conceivably changes over time; and with a health organization to be relied upon for information of untoward events developing concurrently with an abnormality of weather. The immediate endeavor is to develop a reasonable and sensible pattern of assembling the required data, to accomplish its analysis, and to establish principle. The study would profit by extension of observations to other representative climatic regions of the United States.

We are particularly attracted to the value of duplicating such analyses in arctic and tropical zones, where the usual situation is prevailing cold or prevailing heat rather than the seasonal

shifts of temperate regions. The generally incomplete and inadequate data on both weather and deaths limit such observations to selected areas, with the guiding principle that the indigenous and not the casual or foreign population is the concern. Heat waves in such situations have different values than in New York City. Within recent years one of us took part in a whale feast in Wainwright on the arctic coast of Alaska. The day-long festivities ended with dancing, outdoors in the bright light of the arctic summer night, and at a temperature of 42° F. An Eskimo among our friends remarked what a pity it was that we were here at this time of the year; it was just too hot to dance. Similarly cold waves in the jungle tropics have other values, with the resulting influence on frequency of deaths completely speculative.

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# PHYSIOLOGY

PROCEEDINGS OF

## The Physiological Society of Philadelphia

SESSION OF MAY, 23, 1958

### (Joint Meeting of Physiological Society of Philadelphia and Philadelphia Aeromedical Society)

SEMINAR ON HUMAN FACTORS IN EXTRA-ATMOSPHERIC FLIGHT

**Six Man, Seven Day Confinement Study at High Oxygen Concentration.** C. F. GELL, M.D., D.Sc. (MED.), (Air Crew Equipment Laboratory). From April 15 to April 22, six Navy volunteers were confined in a low pressure chamber, designed as a space ship at the Air Crew Equipment Laboratory in Philadelphia. The men were maintained at an altitude of 10,000 feet and breathed oxygen continuously at a pressure 3 1/3 times normal sea level pressure. The purpose of the test was to secure information needed by the bioengineers relative to the highest oxygen concentration in which man could live continuously without developing symptoms of oxygen poisoning. The laboratory is experimenting with a recirculating apparatus for the internal atmosphere of nuclear and space ships. The oxygen source will be from a chemical generator. The test was successfully completed and the knowledge now gained gives the bioengineers a range of 1 to 3 1/3 pressures of oxygen as a safe range in which they may take liberties in developing their equipment.

In this test the men were exposed to rigorous living and a basic minimum of creature comforts. The living space allowed was 65 cubic feet per man. They slept and worked in shifts. Eight hours sleeping, 9½ hours conducting tests of performance, skill and mental awareness and 6½ hours for meals and idling. Their meals consisted of C rations. They were strictly incommunicado and were unable to see outside the chamber. A one way view plate was utilized by observers outside the chamber. The test was entirely successful with the men ending in good physical condition. A mass of data is now being analyzed. The subjects took movies of themselves in the chamber.

**A Unified Concept of Tolerance to Physical Stress: Its Relationship to a System of Aircrew Selection Based on Physiologic and Psychologic Criteria.** TERENCE F. MCGUIRE, CAPT. USAF (MC) (Aero-Medical Lab., WCLDF-3, W.A.D.C., Wright-Patterson AFB). The question of man-to-man variability under acute physical stress was discussed and the following problem areas were examined: a) is there enough man-to-man variability in stress tolerance to be significant, b) is tolerance transferable from one group of stresses to another, and c) how much variability is there within the same man?

The "anatomy" of stress tolerance was briefly discussed and a usable unified concept was presented. The schema encompassed biochemical and endocrine considerations, autonomic nervous system response, and psychic influences.

Utilization of the foregoing informa-

tion led to a program aimed at careful evaluation of personnel for special arduous missions, or for missions where the price of human failure is extremely high. The program thus far has shown such selection to be feasible; the current effort is aimed at rendering it more simple and practical.

**Interactions of Metabolic and Environmental Factors.** C. J. LAMBERTSEN, M.D. (Department of Pharmacology, University of Pennsylvania School of Medicine). In experiments on unanesthetized, normal human subjects, data have been obtained relating to the mechanisms underlying regulation of the central nervous system oxygen, carbon dioxide and hydrogen ion environments, which appear linked through common dependence upon the reactivity of respiratory centers to hydrogen ions. The data presented, while having a bearing upon choice of capsule atmosphere for extra-atmospheric flight, have their chief relationship to basic respiratory control mechanisms. The data suggest that elevation in inspired oxygen tension leads to elevation of central $pCO_2$ and cH, increased pulmonary ventilation, arterial hypocapnia and cerebral vasoconstriction; thus the influence of high inspired oxygen tensions upon mean brain capillary $pO_2$ is self-limiting. Additional data presented indicated: a) that oxygen accomplished cerebral vasoconstriction, not by direct action of oxygen, but through the accompanying arterial hypocapnia, b) that the dilatation of brain vessels by arterial anoxemia could be overcome and even reversed by induced arterial hypocapnia, c) that the depression of brain oxygen consumption produced by inhalation of 8% oxygen could, without raising arterial $pO_2$, be alleviated in part by inducing arterial hypocapnia to improve brain blood flow. The implications of interactions among central control of ventilation, blood gas composition and brain blood flow in determining mean brain capillary $pO_2$ were discussed.

# BOOK REVIEWS AND NOTICES

DIE PERIPHERE INNERVATION. By EMIL VILLIGER. Edited by EUGEN LUDWIG, Universität Basel. 11th ed. Pp. 210; 80 ills. Basel: Benno Schwabe & Co., 1957 (Intercontinental Medical Book Corp., New York, U.S. Agents). Price, $5.00.

THIS concise, well organized description of the peripheral nervous system covers the essential morphological and functional details concerning the innervation of all of the structures taken care of by this portion of the nervous system. It is a straight forward, well organized and very complete presentation of the material. Some very useful tabulations are included for all regions which give the muscles supplied by each nerve and the actions of these muscles allowing one to see at a glance the movements which are affected if a particular nerve is damaged. There is also tabulation of the innervation taken care of by all spinal cord segments. The illustrations serve their purpose well and the majority of them are original diagrammatic presentations which are very graphic. The Nomina Anatomica Parisiensia (1955) has replaced The Jena Nomina Anatomica (1935) of the previous edition. J. H.

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HUMAN HISTOLOGY. By LESLIE BRAINERD AREY, PH.D., Robert Laughlin Rea Professor of Anatomy, Emeritus, Northwestern University. Pp. 337. Philadelphia: W. B. Saunders Company, 1957. Price, $6.50.

THIS book, written by the very capable microanatomist, Dr. Arey, has the rather unique features of being a full-length textbook written entirely in outline form and containing no illustrations. The subject matter basically is divided into three parts: cytology, general histology, and special histology (organology). The relatively concise, yet complete, treatment of general and detailed structure is followed by a practical summary of the "appearance in sections" and "diagnostic features," functional correlations, and regenerative abilities of each tissue and organ. Even though this book is written in a radically different way the subject matter follows the classical interpretation, there being only minor variations.

This book is highly recommended as a reference work and thorough review-source. Its value as a laboratory text would be greatly enhanced by the inclusion of, or access to, appropriate illustrations. J. T.

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MEDICAL TELEOLOGY AND MISCELLANEOUS SUBJECTS. By F. PARKES WEBER, M.A., M.D., F.R.C.P., F.S.A. Pp. 86; no ills. London: H. K. Lewis & Co., Ltd., 1958. Price, 15s.

MOST of the 37 brief articles that make up this slender volume are either reprinted from a medical journal or are excerpts from one of the author's medical articles. The sources of these excerpts are not indicated, thus giving the appearance of notes from a personal diary.

The first part groups 9 articles under the heading Medical Teleology, at best an ill-defined term that here is materially different from such a concept as "adaptation of organic structure adapted to the services of the organism in which they occur." Recognizing the importance of clear definitions, one can regret that Dr. Weber did not tell us the exact meaning of the word as he uses it. The 2 longer articles, a discussion of "Automatic Vital Safety-valve Actions" in disease and "The Blood as a Group of Organs," both deriving from his "Some Thoughts of a Doctor" (H. K. Lewis, 1935), offer, as always with Dr. Weber's writings, food for thought interspersed with appropriate literary quotations. The single page of Introduction is a good example of Dr. Weber's inimitable style: would that it had been longer! Let us hope too that this little collection will not constitute the last production from his prolific pen! E. K.

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PRE-EMPLOYMENT DISABILITY EVALUATION. By WILLIAM A. KELLOGG, M.D., Clinical Professor of Surgery, New York Polyclinic Post-Graduate Medical School. Pp. 120. Springfield: Charles C Thomas, 1957. Price, $10.50.

DR. KELLOGG who was formerly associated with the Medical Department of the Douglas Aircraft Company, Inc., Long Beach, California Division, has performed some 20,000 pre-employment disability evaluations and certainly is well qualified to write such a manual.

The importance of such evaluation of employees is stressed and techniques in exa in-

ing individuals seeking employment are discussed in the first pages of the book. Following this, in a well classified and orderly fashion, numerous disabilities are described succinctly yet adequately. Restrictions are likewise classified and discussed and the restrictions which should be imposed for each disability are defined.

A section on medical forms which might be useful in industrial medicine is included in the closing sections of the book.

It is interesting to note that this manual is constructed so that the pages may be removed along a perforated margin and ring holes are provided for use in the standard three-ring notebook.

This manual is well organized and should provide a ready, reliable reference for the physician concerned with pre-employment disability evaluation. J. M.

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Noise and Your Ear. By Aram Glorig, Jr., M.D. Pp. 152; 28 ills. New York: Grune & Stratton, Inc., 1958. Price, $6.50.

This slim volume, the first of a series of "Modern Monographs in Industrial Medicine," contains an incredible wealth of information concerning hearing and noise. Dr. Aram Glorig, whose work in this area is well known, has performed a genuine service in writing this book. It discusses industrial deafness and related areas clearly and concisely in both the theoretical and practical aspects. Of particular practical use are the "Glossary of Audiological and Medicolegal Terms" and the appendices on organizations and equipment available for studying the problems presented. This book will be of great interest to any practitioner concerned with hearing and will prove to be an indispensable volume on the desk of the industrial physician.

P. R.

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Trace Analysis. Edited by John H. Yoe, M.S., M.A., Ph.D., and Henry J. Koch, Jr., A.B., M.D., University of Virginia and Sloan-Kettering Institute for Cancer Research. Pp. 666; illustrated. New York: John Wiley & Sons, Inc.; and London: Chapman and Hall, Ltd., 1957. Price, $12.00.

A symposium on Trace Analysis was held in November, 1955, at the New York Academy of Medicine, sponsored by the Rockefeller Foundation and the Sloan-Kettering Institute. The present volume consists of papers delivered and discussions arising therefrom.

The speakers and discussants were selected as competent users of, if not authorities on, the procedures described.

The book fulfills a real need for a critical appraisal of research tools used in the analysis of biological material. It should be of value to any worker who uses or contemplates the use of any of the techniques discussed.

Each paper is well illustrated and documented (some references given at the end of each chapter). One might wish for more complete bibliographies, but the papers listed therein are highly pertinent.

The discussions are divided into three sections: (1) Methodology, (2) Instrumentation and (3) Sensitivity, Separation, Concentration and Contamination. R. B.

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Biomicroscopie Du Corps Vitre et Du Fond De L'Oeil. By A. Busacca, H. Goldmann and S. Schiff-Wertheimer. Pp. 382; 144 ills. and 40 plates in color. Paris: Masson et Cie, 1957. Price, 8.000 fr.

This well-illustrated, detailed analysis of the normal and pathologic anatomy of the vitreous and ocular fundus as it is observed by means of the slit lamp biomicroscope represents the results of many years of experience by the authors with this new technique. No other method is as valuable for a detailed study of the vitreous body, and a large number of changes are described and illustrated. Such observations undoubtedly have added significantly to the understanding of the pathologic anatomy and pathogenesis of retinal detachment and other diseases. The possibility of obtaining an optical section of the vitreous, retina and choroid is the principal advantage of this technique when its is used for examination of the ocular fundus. Its value in clinical practice has not been clearly defined in this report. The value of the optical section has been amply demonstrated in the examination of the anterior segment of the eye, however, and its usefulness may ultimately be equally apparent for study of the posterior segment. This section of the book is also abundantly illustrated.

W. F.

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An Atlas of Fetal and Neonatal Histology. By Marie A. Valdés-Dapena, M.D., Associate Professor of Pathology, Woman's Medical College of Pennsylvania. Pp. 200; 237 ills. Philadelphia: J. B. Lippincott Company, 1957. Price, $11.00.

THE purpose of this atlas is to show histologically the development of each of the major organs from 4-months' gestation to early childhood. Even though this purpose generally is fulfilled, there is a paucity of material concerning some of the major organs (for example, only 8 low power photomicrographs of the gastrointestinal tract). An above average number of minor errors detracts from the quality of the book. Among these are incorrect references to photographs on pages 122 and 154 and the misspelling of Hassall's corpuscles on successive pages. This book is recommended, however, for it does present material which previously has not been readily available to all persons concerned with fetal and neonatal histology. J. T.

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YEAR BOOK OF GENERAL SURGERY. Edited by MICHAEL E. DE BAKEY, M.D., Professor of Surgery, Baylor University College of Medicine, and STUART C. CULLEN, M.D., Professor of Surgery, State University of Iowa College of Medicine. Pp. 560; 128 ills. Chicago: Year Book Publishers, Inc., 1957. Price, $7.50.

THIS volume of the Year Book of General Surgery is dedicated to Dr. Evarts A. Graham, who, for many years, edited this volume. Readers in the past had always appreciated the choice of articles and the very appropriate editorial remarks which Dr. Graham had inserted. This reviewer would like to assure prospective readers that Dr. De Bakey has carried out the same wise editorial policy and has presented a book which is a tribute to Dr. Graham.

Abstracts of articles of surgical interest have been arranged into a general section, and, as in previous years, there is an excellent section on anesthesia. As one would expect, there are a large number of articles on cardiovascular surgery, which reflects the interest of the times as well as the interest of the editor.

Once again, this book is recommended to all those having interest in general surgery. By reading it, they will supplement reading done throughout the year and obtain a summary of the important publications of the past year. R. B.

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